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Respiratory Regulation of the Crayfish (Cambarus immunis).

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Some 600 modified Winkler oxygen consumption tests were made to determine (1) the normal variation in oxygen consumption of crayfish under constant oxygen tensions, CO_2 content and H-ion concentration, (2) the effect of H-ion concentration on oxygen consumption, and (3) the oxygen consumption under successively lowered oxygen tensions.

Crayfish were found to exhibit considerable variation in their normal oxygen consumption from hour to hour, the consumption being frequently doubled or halved. Both sexes, in weights ranging from 1.5 to 28.0 gm., exhibit this respiratory fluctuation.

Oxygen consumption determinations for 6 consecutive hours, during which crayfish were tested in water ranging in pH from 6.8 to 5.2, gave no evidence within these limits of a possible effect of H-ion concentration on respiration. The variation in oxygen consumption from hour to hour was of the same magnitude previously determined for normal variation in oxygen consumption under a constant pH.

Respiratory regulation under different oxygen tensions was determined by measuring the oxygen consumption, for successive hours, of crayfish which were steadily lowering the oxygen tension of the water by means of their own respiration. Crayfish were tested individually and in groups of 10. The range of oxygen tensions investigated was from 115% to 8% saturation, partial pres-

sure, at 25° C. Grouping the data according to the weights of the individuals revealed that large animals averaging 17.1 gm. were able to regulate oxygen consumption in a normal manner, down to about 40% saturation. Medium-sized animals, averaging 9.0 gm. regulate down to about 30% saturation, while smaller individuals averaging 4.3 gm. are able to regulate down to about 20% saturation.

Asphyxiation occurs in definite stages and is initiated between the tensions of 15 and 10% saturation. Shortly before or during asphyxiation, the crayfish frequently liberates oxygen, which phenomenon is generally followed by increased respiration. Upon death, oxygen is liberated by the body.

Respiratory regulation in the crayfish is very good down to between 40 and 20% saturation, depending on the age of the individual. Below the lower limit of regulation, respiration is spasmodic and is followed shortly by asphyxiation. Within the limits tested, CO_2 content and H-ion concentration of the water have no marked effect on oxygen consumption. Finally, the crayfish normally exhibits considerable hour by hour variation in oxygen consumption.

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Influence of Annular Tympanic Cartilage on Development of Tympanic Membrane (Rana pipiens).

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The formation of the tympanic membrane constitutes one of the last adult structures to be developed during the metamorphosis of the frog tadpole. The present communication, based on over 300 autoplastic transplantations, is designed to point out the factors responsible for its formation.

Histological sections of tympanic membranes in various stages of development show clearly that a definite series of events takes place in the integument during which the *stratum spongiosum* and *stratum compactum* layers disappear and the latter is replaced with a network of fibrous elements typical of the adult tympanic membrane.

Preliminary transplantations of skin grafts from the back and side of the tadpole to the tympanic membrane region, resulted in the formation of typical membranes in the grafts during metamorphosis.

This would indicate that all integument of the metamorphosing tadpole is totipotent in this regard and that structures in the immediate vicinity of the tympanic membrane region have a direct influence on membrane formation.

Autoplastic transplantations of the annular tympanic cartilage in various stages of development were made to regions under the skin of the side and under the skin of the back of metamorphosing tadpoles. Typical tympanic membranes were formed in the skin of the back directly over the annular tympanic cartilage transplants. Less perfectly formed membranes developed in the skin of the side directly over the transplants. Histological sections of such membranes showed that typical cellular transformations had taken place, identical to those found in normal membrane formation.

All cases of complete extirpation of the annular tympanic cartilage exhibited a total absence of tympanic membrane formation, all external and histological evidences being absent. This, together with the transplantation results, indicates the necessary presence of the developing annular tympanic cartilage for normal tympanic membrane formation.

Transplantation of the annular tympanic cartilage beneath normal integument at a late stage of development, and at a time when the normal tympanic membrane is well formed, induced slight, if any, transformation of the integument. This would indicate that the influence of the annular tympanic cartilage, on membrane formation, is limited to a definite period of its development. The data indicate also that skin will transform into tympanic membrane only when in close contact with the annular tympanic cartilage. The formation of the normal tympanic membrane illustrates an indirect method of the thyroid hormone in bringing about certain metamorphic transformations in the tadpole.

This conclusion seems justified in view of the fact that the skin fails to undergo modifications typical of tympanic membrane formation in the absence of the annular tympanic cartilage. If the thyroid hormone operated directly through the blood stream, removal of the annular tympanic cartilage would not have inhibited the process. It is only indirectly, therefore, through the development and presence of the annular tympanic cartilage, that the thyroid hormone is able to induce tympanic membrane changes in the skin over the cartilage.

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Evidence of Direct Hormonic Influence on Growth and Differentiation of Frog's Tongue During Metamorphosis.

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During metamorphosis, the tongue anlage of the tadpole undergoes rapid growth and differentiation. Autoplastic transplantations of the anlage to the musculature of the back of metamorphosing and normal *Rana pipiens* tadpoles were made, to determine whether normally adjacent structures of the mouth influence the development of the tongue, or whether differentiation is due to direct hormonic (thyroid) control.

Growth of the anlage transplanted to the back was apparent in over 90% of the cases during metamorphosis, and produced distinct bulging of the integument of the back. In many cases growth resulted in the formation of tissue 8 to 12 times greater in volume than that of the original anlage. The tissue masses were generally oval in shape.

Histological sections of the grafts exhibited the differentiation of typical tongue elements to a considerable extent. These included the formation of a mucous membrane containing both filiform and fungiform papillae. Muscle fibers and mucous glands were also fully developed. All grafts appeared fairly well vascularized and in some, slight movement or twitching occurred indicating, possibly, some degree of innervation.

Macroscopically, the grafts never attained the typical elongated and forked appearance of the normal tongue. This was probably due to a decreased rate of growth and differentiation, coupled with the limited time available for growth before the death of the animal at the close of metamorphosis.

The results indicate that growth and differentiation of the tongue is controlled directly through hormonic influences (probably of the thyroid gland) operating through the blood supply to the anlage. This seems logical in view of the fact that the tongue anlage requires only an adequate blood supply for growth and differentiation, and is not dependent on the possible influence of normally adjacent structures of the buccal cavity. Failure of growth or differentiation when transplanted to other regions would have strongly indicated a secondary influence. Since the reverse was found to be true, however, direct hormonic influence through the blood stream would appear to be responsible.

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Further Evidence of Destruction of Vitamin B in Evaporated Milk.

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Hartwell's[1] method of determining the Vitamin B content of foods differs from that of other workers, in that it consists in measuring the effects of a given ration on the suckling young. Either just before or just following parturition the mother animal is put on a high protein ration containing all the known necessary constituents, with the exception of Vitamin B. In the absence of an adequate amount of Vitamin B the suckling young gain normally for about 13 days, when they suddenly develop convulsions, manifested by general muscular incoordination. This condition is frequently accompanied by unusual outcries, and dragging of the hind legs. The baby animal gasps for breath, and finally dies within a few hours. That these animals do not die of insufficient food is indicated by the fact that milk is very frequently found in the stomachs. When adequate amounts of Vitamin B are added to the ration the young animals develop normally and are weaned at the usual time.

By this method,[2] comparative studies of fresh and evaporated milks have indicated that evaporated milk is quite deficient in Vitamin B. Since these findings are not in accord with results reported by Gibson[3] on pigeons, dogs, and pigs, nor with the recent work of Dutcher,[4] arrived at by the more usual method of feeding the growing young directly, it seemed possible that the untoward symptoms in Hartwell's animals might be caused by a calcium deficiency, or a lack of Vitamin D. Previous experiments showed that in the process of evaporation the calcium and phosphorus in milk is made less available.[5] We have, therefore, repeated Hartwell's experiments, making certain additions and modifications in the ration. In our experiments the pregnant animals, which had received our stock ration throughout the gestation period, were given the experimental ration just previous to parturition. When the young were 4 days old all but 4 of the litter were killed. From this time the suckling young were weighed every 4 days.

The ration modifications and the results of the experiments are given in Table I.

TABLE I.

Comparison of Whole and Evaporated Milk Rations as a Source of Vitamin B for the Suckling Young.

Ration	Group	Wt. of litter 4 days	Age at onset of spasms	Wt. at onset of spasms	Ave. wt. of young at 1 mo.	Total No. of young weaned
		gm.	days	gm.	gm.	
Control[1]	A	24	13	62+		0
	B	26	12	72		0
100 cc. of whole milk substituted for 50 cc. evaporated milk	A	20			52	4
Control + 0.2 gm. $Ca_3 (PO_4)_2$	A	37	14	100	41	1
	B	28	15	93		0
Control + 1 gm. $Ca_3 (PO_4)_2$ + 5 cc. cod liver oil	A	39	13	94		0
Irradiated evaporated milk used in ration	A	29	13	64		0
	B	21	14	60		0
Control + 0.2 gm. $Ca_3 PO_4)_2$ + 20 cc. wheat embryo extract[2]	A	33			42	3[4]
	B	33			50	4
Control + 30 cc. wheat embryo extract[3]	A	39			45	5[5]

[1] This ration consisted of 30 gm. of dried wheat bread, 12 gm. of casein, 50 cc. of evaporated milk, .05 cc. ferric citrate (6% solution) and 0.1 cc. of potassium iodide (2% solution).

[2] Alcoholic extract of 10 gm. of wheat embryo.

[3] Alcoholic extract of 15 gm. of wheat embryo.

[4] One died at 21 days. Cause unknown.

[5] This animal suckled 6 young. One was lost at 3 weeks.

Neither the addition of calcium phosphate nor of cod liver oil to the evaporated milk ration, nor the substitution of irradiated evaporated milk modified the untoward symptoms in the young; whereas the addition of wheat embryo extract to the evaporated milk ration, or the substitution of quickly boiled milk for evaporated milk resulted in normal young. It would, therefore, appear that some substance in milk is destroyed by the evaporation processes. This substance is contained in the wheat embryo extract.

These results seem to be verified by tests with pigeons.

The daily administration of 50 cc. of raw milk, when given in conjunction with 20 grams of polished rice was found sufficient to protect pigeons against polyneuritis; whereas 25 cc. of evaporated milk was insufficient. When the amount of evaporated milk was increased to 30 cc. the untoward symptoms were only slightly manifest.

These results are less striking than those with the suckling rats. It is possible that the very young are more sensitive to an antineuritic deficiency. The experiences with beri-beri in the nursing infant are comparable.

Sherman and Axtmayer[6] and Chick and Rosco[7] have recently

TABLE II.

Polyneuritic Symptoms in Pigeons Receiving Evaporated Milk and Fresh Milk.

Initial Wt.	Forced feeding	Milk* Kind	Amt. Milk	Untoward Symptoms	Gain in Wt.	
gm.	days		cc.		gm.	days
235	33	Fresh	50		65	33[1]
265	20	Evap. + 1 gm. $Ca_3(PO_4)_2$	25	Unable to stand. Regurgitates food. Head hangs on side. Crop hard.	32	30[2]
262	22	Evap. + 1 gm. $Ca_3(PO_4)_2$	25	Food not retained. Unable to swallow. Breathing heavily; head down.	5	22[3]
270	30	Evap.	30	Tired; unable to stand erect; crop soft	50	30[4]
302	30	Fresh	60		68	30[5]
	30	Fresh	60			6

* During forced feeding period each bird was given daily in addition to milk 20 gm. polished rice. Gravel available in pens.

[1] Pigeon vigorous, active. No signs of polyneuritis.

[2] Given 15 cc. wheat embryo extract subcutaneously. Following day bird very much alive; active; crop soft.

[3] Bird died. Enlargement of heart questionable; no pericardial fluid.

[4] Less vigorous than Pigeon 5, but no outstanding signs of polyneuritis.

[5] Pigeon vigorous; active. No signs of polyneuritis.

[6] Pigeon stands erect; walks, flies; no signs of polyneuritis.

shown that Vitamin B contains both an antineuritic substance, Vitamin F, and a growth promoting factor, Vitamin G, the former being less thermostable than the latter. Milk is reported as being richer in Vitamin G than it is in F. Our results would seem to indicate that the antineuritic factor is destroyed in part in the evaporated milk.

[1] Hartwell, G. A., *Biol. Chem. J.*, 1922, xvi, 825.

[2] Hartwell, G. A., *Biol. Chem. J.*, 1925, xix, 227.

[3] Gibson, R. B., and Concepcion, I., *Philippine J. Sc. B.*, 1916, xi, 119.

[4] Dutcher, R. A., Frances, E., and Combs, W. E., *J. Dairy Sc.*, 1926, ix, 379.

[5] Daniels, A. L., and Laughlin, R., *J. Biol. Chem.*, 1920, xliv, 380.

[6] Sherman, H. C., and Axtmayer, J. H., *J. Biol. Chem.*, 1927, lxxv, 207.

[7] Chich, H., and Rosco, M. H., *Biol. Chem. J.*, 1927, xxi, 698.

3751

Temperature Coefficients for Development in Cladocerans.

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In an earlier paper[1] it was shown that the temperature characteristics for the duration of an adult instar in several species of cladocerans were of the same order of magnitude as those found for development in other arthropods, but that certain specific differences were still markedly manifest. The temperature characteristics for the different temperature ranges and also the position of the critical temperatures or "breaks" in the *log rate* against *reciprocal absolute temperature* graphs were accorded a natural interpretation in terms of the geographical distribution and seasonal rhythms of the species studied. The present paper presents data on the temperature coefficients for the complete parthenogenetic generation cycle of several common species of cladocerans.

The literature on the biological application of van't Hoff's rule has been summarized by Kanitz[2] and Przibram.[3] Most of the papers dealing with this subject have been concerned with the relative magnitudes of the temperature coefficients and a subsequent classifying of vital activities into those controlled by physical forces and those controlled by purely chemical reactions. The comparison of several species of cladocerans reared in the laboratory for many generations under similar environmental conditions should reveal specific differences, should such be present, and might be taken to suggest a means for the physiological comparison of related forms. In addition to the ease of propagation in the laboratory, cladocerans are well adapted for such a study as their parthogenetic reproduction insures material that is genetically uniform, and their development by instars provides stages which are morphologically well defined.

Six species of cladocerans were used and one of these, *Daphnia pulex*, included two varieties, the typical form and a thelytokous form[4] which is probably hexaploid in constitution.[5] The following table gives the time in hours from the beginning of the first young instar until the end of the first adult instar, when the females produce their first brood of young, for each of several temperatures and the Q_{10} for the temperature intervals 13° to 20°, 20° to 30°, and 25° to 35° C.

On comparing the Q_{10} of the different species for the temperature interval 20 to 30°, decided differences are apparent. Two of

TABLE I.

°C.	Moina macrocopa		Pseudosida bidentata		Simocephalus, all species		Daphnia pulex (type)		Daphnia longispina		Daphnia magna		Daphnia pulex (984)	
	time in hours	Q_{10}	time in hours	Q_{10}	time in hours	Q_{10}	time in hours	Q_{10}	time in hours	Q_{10}	time in hours	Q_{10}	time in hours	Q_{10}
13	296.8		517.6		381.0		309.7		284.5					
		3.96		2.90		2.36		2.64		1.82				
20	114.2		246.0		208.5		156.8		187.0		203.1		170.3	
25	66.9	2.39	134.3	2.03	129.1	1.81	100.8	1.70	138.0	1.48	146.0	1.31	120.4	1.19
30	49.5	1.76	121.2	1.63	115.0	1.15	92.1		126.0		155.0		143.2	
35	38.0		82.4		112.5		Will not live and reproduce.							

the species show a value of the temperature coefficient which is above 2.00; two with a value near 1.75; one with a value near 1.50; and two with a value near 1.25. The reality of these differences indicated by the temperature coefficient for development is emphasized by comparing the lethal temperatures of the adults of these same species. The lethal temperatures, determined arbitrarily by suddenly immersing the animals in water of the desired temperature for one minute, are: *M. macrocopa* 48°, *P. bidentata* 48°, *Simocephalus* species 43°, *D. pulex* typical 44°, *D. longispina* 42°, *D. magna* 41°, and *D. pulex* "984" 41°. The species are seen to fall approximately into the same groups as determined by the temperature coefficient for development of the younger animals.

[1] Brown, L. A., *J. Gen. Physiol.*, 1926-27, x, 111.

[2] Kanitz, A., *Temperatur und Lebensvorgänge*, 1915, Berlin.

[3] Przibram, H., *Temperatur und Temperatoren*, 1923, Leipiz und Wein.

[4] Banta, A. M., *Z. Indukt. abstammungs u. vererbungl.* 1925, xl, 28.

[5] Schrader, F., *Z. Indukt. abstammungs u. vererbungl.*, 1925, xl, 1.

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The Effect of CO_2 Administration Upon Parathyroid Tetany.

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It has been known for some years that certain mineral acids such as HCl, and acid producing salts such as $CaCl_2$ and NH_4Cl are effective agents in the treatment of infantile tetany, and have also been successfully employed in cases of experimental tetany.

The effect of injecting HCl and $CaCl_2$ or NH_4Cl is the production of an acidosis. It seems from a survey of the literature on the subject, that the gross manifestations of tetany, even the most violent convulsions, are rather promptly relieved by slight changes in the reaction of the blood toward the acid side, and that such changes influence in some manner the calcium content of the blood.

In view of these facts it was considered worthwhile to test the effect of administering CO_2 to dogs in tetany, (1) in relation to relief of symptoms and (2) the effect upon the serum calcium level.

Small rat and fox terriers were used as experimental animals. They were thyroparathyroidectomized and permitted to develop tetany. When the animals were prostrate they were bled and the serum Ca, CO_2 capacity, CO_2 content and pH determined. Following bleeding, the dogs were immediately placed in a specially constructed CO_2 chamber and sufficient gas administered over a 20 to 40 minute interval to produce unconsciousness.

The rapidity with which the muscle tremors, jerks and convulsions disappear following CO_2 treatment is remarkable. Within a few minutes following treatment the animal shows a complete return to norm. The length of the recovery period varies considerably. Some animals do not again exhibit tetany symptoms for 12 hours, others show tetany within a few hours. If such animals are again placed in the CO_2 chamber they again temporarily recover. No effort was made to keep the animals alive indefinitely, since we were primarily interested in the effects produced by the acid intoxication.

The dogs were again bled for Ca, CO_2 capacity, CO_2 content and pH after removal from the CO_2 chamber. The data showed no change in the serum Ca but a very marked drop in the CO_2 capacity, CO_2 content, and pH of the blood, evidence indicative of acid intoxication. It was evident from the CO_2 change in the blood that the acidosis was not due to the CO_2 *per se,* but probably to a rise in organic acid. Since the CO_2 treatment was really a simple method of asphyxiation it seemed probable that lactic acid was the responsible agent.

Further investigation showed the surmise to be correct. The rise in the blood lactic acid following CO_2 treatment is striking and consistent, and in our opinion is sufficient to account for the degree of acidosis observed. The normal lactic acid content of both normal and operated dogs in tetany is approximately 27.5 mg. per 100 cc. Following recovery from tetany after CO_2 treatment lactic acid values of over 100 mg. per 100 cc. were encountered. The decreased CO_2 capacity and CO_2 content of the blood of CO_2 treated dogs, is obviously due to the increase in lactic acid with consequent blowing

off of CO_2. The data from one of our experimental dogs are given below.

TABLE I.

Oct. 30	Lactic acid mg. %	CO_2 content vols. %	CO_2 capacity vols. %	pH	Remarks
10:00 A. M.	28.7	33.3	43.9	7.44	Violent tetany
10:30 A. M.	56.5	22.2	27.6	7.25	CO_2 for 20 min Recovery
3:40 P. M.	27.9	35.0	42.7	7.45	Tetany
4:05 P. M.	88.9	29.2	32.7	7.29	CO_2 for 20 min Recovery

Following return of the lactic acid to a normal level, *i. e.*, (about 27.5 mg. per 100 cc. blood) tetany symptoms again appear. The evidence at hand seems to indicate that the change in the blood reaction toward the acid side relieves tetany by rendering serum Ca more diffusible, and also probably stimulates excretion of the excess phosphorus. The total serum Ca in all of our animals remained unchanged by the CO_2 treatment, despite the fact that the animal appeared normal and was free from tetany.

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Survival Period in the Pregnant and Lactating Cat Following Adrenal Extirpation.

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H. A. Stewart[1] first called attention to what he considered to be an unusual prolongation of the life-span of the pregnant cat following adrenal removal. He states "In most instances the pregnant and lactating animals survived the extirpation of the adrenal glands longer than normal, non-pregnant or male animals." The average prolongation in good cases of non-injected pregnant cats was 7.2 days, whereas the average prolongation of male and non-pregnant females was only 2.2 days.

Stewart and Rogoff,[2] working with dogs, cited cases of pregnant bitches which lived for considerable periods following bilateral adrenal removal. Several animals survived throughout the gestation period, and one animal gave birth to a litter of pups. These authors do not attempt to explain the reason for such prolongation of the survival period of adrenalectomized dogs, but merely suggest that possibly the *corpus luteum* may be involved.

In an earlier series of experiments on bilaterally adrenalectomized cats,[3] the writer observed that pregnant individuals did not survive any longer than male or non-pregnant animals. The observations at that time were limited to only a few cases, hence no significance was attached to them. During the fall and spring of 1927, however, the problem of the survival period of double-operated, pregnant cats was carefully studied. Sixteen female animals were operated under anesthesia and their survival periods were checked against those of 6 operated male and non-pregnant female animals which served as controls.

All animals were operated with a 3 to 17-day interval between the removal of the right and the left adrenal glands. They were kept at a temperature of approximately 22° C. and were permitted the freedom of the animal room. Food was at all times available to them but no attempt was made to feed them forcibly, following the onset of anorexia which invariably succeeds (50-58 hours) the removal of the glands. All animals eventually died in coma, following the typical syndrome attendant upon the extirpation of both adrenals. At death each animal was carefully autopsied and histological sections were made of the ovaries in those cases in which any doubt existed as to whether an intervening ovarian cycle had taken place since absorption or resorption, where such was indicated by the condition of the uterine walls.

The average life-span of untreated, bilaterally operated cats is approximately 100 to 120 hours from the time the second adrenal is removed. If the animals are forcibly fed, or injected with considerable quantities of fluids, the survival period is greatly increased.

Sixteen cats in various stages of pregnancy were employed. The animals were always autopsied at death and the number and measurements of the foetuses recorded. Six male and non-pregnant females operated at the same time, and kept under identical conditions, served as controls. The results of the experiment are as follows: (1) The presence of the adrenal cortex has little or no ameliorative effect upon the syndrome following adrenal extirpation of the mother. (2) The survival period of bilaterally operated pregnant cats is approximately the same as that of male and non-pregnant female animals similarly operated. (3) The mechanism present in the dog which enables the adrenalectomized mother to live throughout the period of gestation is apparently lacking in the cat.

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[1] Stewart, H. A., 17th International Congress of Medicine, London, 1913, Sec. III.

[2] Stewart, G. M., and Rogoff, J. M., *Am. J. Physiol.*, 1927, lxxix, 508.

[3] Corey, E. L., *Am. J. Physiol.*, 1927, lxix, 633.

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Sulfate Retention in Dogs Following Bilateral Adrenal Extirpation.

W. W. SWINGLE AND W. F. WENNER.

From the Zoological Laboratory, State University of Iowa.

The results of previous work by the senior writer[1] and his co-workers[2] on the functional significance of the suprarenal cortex, indicated that the cause of death in double operated cats and dogs is probably acid intoxication, due to retention of acids formed in the course of normal metabolism. The acidosis is apparently not due to the formation of abnormal acids within the organism, but due to defective elimination of non-volatile acids.

Study of the acid-base equilibrium of normal and bilaterally adrenalectomized cats showed quite clearly that the animals with marked symptoms of adrenal insufficiency were suffering from an uncompensated non-volatile acidosis. According to the data available, the acidosis was attributed to an increase in phosphoric and undetermined organic acids. However, it was evident that the marked fall in bicarbonate, observed in our double operated animals, was not occasioned to any considerable extent by the rise in inorganic phosphorus, which although marked, never exceeded two millimols, whereas the carbon-dioxide fell from 6 to 10 millimols. We stated that "increase in organic acid (which in our data included the sulfate ion) is the one change of sufficient magnitude to be responsible for the bicarbonate fall. Further efforts on our part to determine the nature of the organic acid or acids ended in failure, hence it was decided to test for sulfate retention, to see if the sulfate ion could be responsible for the observed changes.

Dogs were used as experimental animals, and only those animals surviving 4 to 6 days or longer, following the second operation, were employed for blood tests.* The results obtained are of interest. The blood of normal unoperated and unilaterally operated dogs have inorganic sulfate values averaging 2 mg. per 100 cc. Double-operated animals, showing no symptoms of adrenal insufficiency, also have normal amounts of blood inorganic sulfate. However, when serious symptoms of adrenal insufficiency intervene, the rise in inorganic sulfate becomes apparent and increases up to the time of death. Values as high as 12 mg. sulfur per 100 cc. of blood are not uncommon. The increase is most marked when the animal is verging on coma. Inorganic phosphate and inorganic sulfate be-

* All operations under careful anesthesia.

have similarly with respect to the stage of adrenal insufficiency during which the greatest increases occur. Increased concentration of inorganic phosphate and sulfate in the blood parallels the increase in severity of the symptoms and the degree of acidosis. Some of the data obtained from 6 of our 25 cases are presented in Table I.

TABLE I.

Sulfate retention in adrenalectomized dogs.

Unilaterally operated		Sulfur mg. per 100 cc.	Bilaterally operated	1st symptoms noticed	Survival period	Sulfur per 100 cc.	pH	CO_2 capacity vols. %	CO_2 content vols. %	P mg.	Sugar, mg. per 100 cc. mg.	Condition animal when bled
Mo. day	hr.											
6/8 at 10	A.M.	0.9 mg.	6/15 at 4 P.M.	6/18 at 1 P.M.	5 days	11.9	7.26	27.	20.6	10.	56	weak
6/9 at 11	A.M.	1.2 "	6/16 at 2 P.M.	6/19 at 9 P.M.	6 "	10.7	7.25	25.	19.2	9.2	50	weak
5/18 at 2	P.M.	1.4 "	5/26 at 9 A.M.	5/29 at 10 A.M.	5½ "	11.1	7.22	22.	19.0	12.1	45	verging on coma
5/21 at 3	P.M.	1.2 "	5/29 at 3 P.M.	6/2 at 11 A.M.	5 "	9.4	7.27	27.	21.5	13.1	57	weak
5/24 at 11	A.M.	1.2 "	5/30 at 9 A.M.	6/3 at 8 A.M.	6½ "	12.2	7.25	26.	21.4	10.2	62	weak
5/20, 10	A.M.*	1.4 "	5/28 at 5 P.M.	5/31 at 2 P.M.	4½ "	9.1	7.27	32.1	28.1	12.5	53	weak

* Venous blood used.

It is evident from our data that retention of the sulfate ion plays a considerable rôle in the acid intoxication of adrenal insufficiency. Whether or not the rise in inorganic phosphorus and sulfate are sufficient to account entirely for the degree of acidosis observed, the writers are not prepared to say. It seems likely that the fall in CO_2 is to a considerable extent due to retention of these two acids. There is also probably a slight increase in organic acid or acids of undetermined nature.

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[1] Swingle, W. W., and Eisenman, A., *Am. J. Physiol.*, 1927, lxxix.

[2] Corey, E. L., *Am. J. Physiol.*, 1927, lxxix, 633.

[3] Zwemer, R. L., *Am. J. Physiol.*, 1927, lxxix.

Missouri Branch.

St. Louis University School of Medicine, November 16, 1927.

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Further Studies on the Emptying of the Gallbladder.

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(Introduced by E. A. Graham.)

From the Department of Surgery, Washington University and Barnes Hospital.

In previous publications[1, 2] on the filling and emptying of the gallbladder we came to the conclusion that two factors were especially concerned in the emptying process, namely, the ebb and flow of the bile from the liver, and elastic recoil of the gallbladder wall. While intrinsic muscular contractions were believed to play some part, they could not at that time be demonstrated.

The observations of Boyden,[3] and of Sossman, Whitaker and Edson[4] that following the administration of a fatty meal, preferably of egg-yolk and cream, the gallbladder is frequently found in a collapsed state, has shed further light on this problem, which we have reinvestigated in the experiments reported here.

We have used the method adopted by Whitaker for experimental animals. The gallbladder is exposed at operation and filled with iodized oil (iodipin) and 24 hours later the animal is recovered sufficiently for the experiment. It is then found that if the animal is starved no emptying of the gallbladder occurs for 2, 3, or more days. A meal of egg-yolk and cream, however, causes a fairly rapid discharge of the iodized oil from the gallbladder along the common duct into the duodenum. This emptying ceases usually after 3 or 4 hours at the end of which time the gallbladder, in the cat, may be completely empty. More often traces of the iodized oil are left in the gallbladder, and these may remain until a further meal is ingested.

To exclude elasticity and variations of intra-abdominal pressure, iodized oil was placed in the gallbladder of a cat, and a canula connected with a finger cot was inserted into the cystic duct. This procedure allowed free communication between the gallbladder and the rubber bag. Twenty-four hours after operation the gallbladder

was still full in spite of the fact that elasticity and variations of intra-abdominal pressure had had full play. A fatty meal was given and in 5 hours a considerable quantity of the iodized oil had passed from the gallbladder into the rubber bag. This experiment indicates that a part of the emptying process is due to muscular activity of the gallbladder wall. Other experiments, likewise, demonstrate that changes of intra-abdominal pressure play a minor rôle in the emptying of the viscus. We believe, however, that muscular contraction is not the only factor involved, and that elasticity and the ebb and flow of fresh bile from the liver play an important part.

The presence of pancreatic juice was found necessary for the fatty meal to cause emptying of the gallbladder. The intravenous injection of secretin, insulin and pancreatic juice did not produce a discharge of iodized oil from the gallbladder, nor did alcoholic or acid extracts of the gallbladder wall.

Although no one has observed contractions of the common duct in the human, it is of interest that they can be easily observed in the pigeon. They are of definite peristaltic nature, being preceded by a relaxation phase which travels rapidly down the duct. Iodized oil passes from the common duct of the cat, even if the hepatic and cystic ducts are ligated. This may possibly be due to muscular contraction.

It is well known that water, iron salts, and a few other substances are absorbed by the wall of the gallbladder. We have shown that sodium iodide is rapidly absorbed. This can be demonstrated by the X-ray.

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[1] Copher, Glover H., Kodama, Schuichi, and Graham, Evarts A., *J. Exp. Med.*, 1926, xliv, 70.

[2] Graham, E. A., *Surg., Gyn. and Obst.*, 1927, xliv, 153.

[3] Boyden, E. A., *Anat. Rec.*, 1926, xxx, 333-363.

[4] Sossman, M. C., Whitaker, L. R., and Edson, P. J., *Am. J. Roent, and Rad. Therap.*, 1925, xiv, 495.

3756

The Blood Amylase in Pancreatic Disease.

ROBERT ELMAN. (Introduced by E. A. Graham.)

From the Department of Surgery, Washington University and Barnes Hospital.

The blood amylase of a number of patients was studied, including a few in which pancreatic disease was suspected from the abnormal amylase findings. In each case the suspicion was verified by the

finding of definite pancreatic disease at operation. In 30 patients convalescent from or suffering from other diseases the blood amylase was found to be rather uniform, the values varying between 4, 5 and 6.0 units.

The method used in measuring the amount of amylase in the blood has been described elsewhere[1] and involved the use of the viscosimeter. One variation was found necessary. A new batch of starch was found to yield a more viscous solution, so that to prepare a suitable fluid for use in the viscosimeter a 3% solution was found appropriate instead of 7%, as previously described. The relative values remained unchanged, though comparison between these values and those already published for dogs cannot be made.

TABLE I.
Blood Amylase in Patients with Pancreatic Disease.

Pt.	Age	Amylase units	Diagnosis	Remarks
J. C.	55	0.5	Ca Pancreas	Marked jaundice. Hard growth involving head of pancreas and atrophy of rest of gland. Cholecystenterostomy done.
W. F.	31	7.8	Chr. Pancreatitis	Marked jaundice.
		25.0		Two weeks later; jaundice much less. Operation revealed chronic cholecystitis and a hard indurated pancreas.
R. G.	28	15.0	Pancreatic Cyst	Large epigastric tumor. Marsupialization of cyst at operation with evacuation of 2 liters of clear fluid containing 300 units of amylase.
		6.1		4 days post op. Recovery uneventful.
		4.5		Six months later. Operation for gall stones showed pancreas small and somewhat hard.
A. F.	45	50.0	Pancreatic Cyst	Large epigastric tumor. Marsupialization at operation with evacuation of 3 liters of dark brown odorless fluid. Content of amylase 1200 units.
		10.4		Two weeks post op. Recovery uneventful.
P. S.	27	23.	Acute Pancreatitis	Two day post op. Fat necrosis at operation.

The amylase content of the blood in two cases of pancreatic cyst, one case of acute pancreatitis, one case of cancer of the pancreas and one case of chronic pancreatitis was studied. Two of these cases had jaundice. This in itself is incapable of influencing blood amylase, for 5 other cases of jaundice due to common duct obstruction all yielded normal values.

[1] Elman, R., and McCaughan, J. M., *Arch. Int. Med.*, 1927, xl, 58.

3757

Some Effects of Borate Upon Reducing Sugars.

MILTON LEVY AND EDWARD A. DOISY.

From the Department of Biological Chemistry, St. Louis University School of Medicine.

In a study of urines from diabetic dogs, it was noted that boric acid interferes with the determination of glucose by Folin's[1] method. Losses of 7.5% to 13% were found in 2 hour urines to which saturated boric acid solution was added. Other alkaline copper methods also gave low values with glucose solutions containing boric acid as well as with fructose, galactose, maltose and lactose, but not with arabinose. Boric acid does not interfere with the oxidation of glucose by Barfoed's reagent.

The oxidation of glucose, galactose, arabinose and maltose by alkaline iodine solutions is retarded by the presence of borate.

The low specific rotation of glucose in the presence of borax observed by Rimbach and Weber[2] and by Murchhauser[3] is due to the formation of an ester between the borate ion and glucose which decomposes on the addition of a strong acid to give an excess of α glucose. This mutarotates to the normal equilibrium value.

[1] Folin, O., and Peck, E. C., *J. Biol. Chem.*, 1919, xxxviii, 287.

[2] Rimbach, E., and Weber, O., *Z. f. Physikal. Chem.*, 1905, li, 477.

[3] Murchhauser, H., *Biochem. Z.*, 1923, cxxxviii, 6.

3758

Myostatic Contracture and Other Changes in the Extensibility of Skeletal Muscle.

S. W. RANSON AND H. H. DIXON. (Introduced by Leo Loeb.)

From the Department of Neuroanatomy, Washington University Medical School.

By myostatic contracture we mean to designate a condition of permanent shortening in resting muscle, which is maintained in the entire absence of nerve impulses, the muscle having acquired, usually as the result of prolonged immobility, a new and shorter than normal resting length. Familiar examples are the contractures which limit the movement of joints after prolonged immobilization in plaster casts, the permanent shortening of muscles after division of their

tendons, the paretic contractures due to the unequal paralysis of antagonistic muscle groups in anterior poliomyelitis and multiple neuritis and the rigidity seen in the latter stages of local tetanus.

Muscles in myostatic contracture have approximately normal elasticity, but their ductility, *i. e.*, their tendency to undergo permanent elongation when stretched, is greatly reduced.

The muscle tested was the gastrocnemius of the white rat with sciatic nerve cut but circulation intact. The muscle was stretched with loads increasing by 10 gm. intervals with 2 minute cycles of stretching and rest. A weight of 10 gm. was applied for 1 minute and removed for 1 minute, then 20 gm. was applied for 1 minute and removed for 1 minute and so on up to 100 gm.

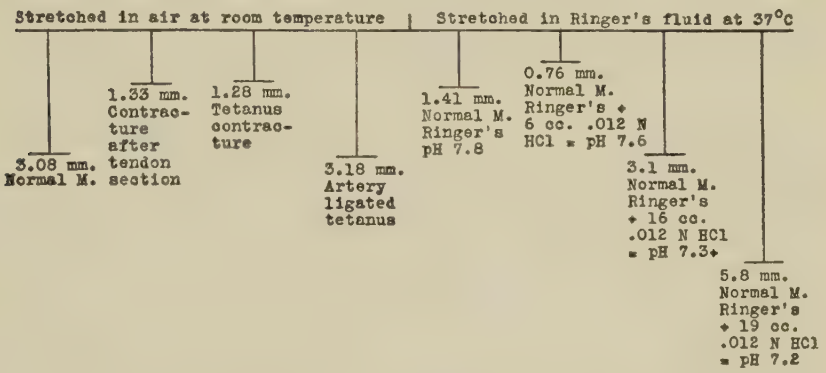
In the first set of experiments the muscles were exposed to the air at room temperature while being stretched. Under these conditions a series of 10 normal muscles gave an average permanent elongation of 3.08 mm.; 10 muscles in contracture 15 days after section of the *tendo achillis* gave an average permanent elongation of 1.33 mm.; and 9 muscles in tetanus contracture gave an average permanent elongation of 1.28 mm. This marked decrease in ductility is not due to fibrosis. Histological sections of these muscles show no increase in connective tissue and only slight changes in the muscle fibers, such as a blurring of the cross striations. There are no structural changes which could account for the decreased ductility. It is possible that there may be an accumulation of lactic acid, and preparations are being made to determine the amount of this substance in these muscles. But it would seem that the contracture depends on some more subtle chemico-physical change in the muscle; because ligation of the artery, supplying a muscle in tetanus contracture will usually, though not always cause a sudden and marked though rather transient increase in its ductility. A suggestion of how the increased acidity which follows in the wake of the anemia might cause a relaxation is given by the next series of experiments.

In order to meet the obvious objections against working with a muscle exposed to the air at room temperature, other experiments were conducted with the muscle immersed in Ringer's solution at 37° C. This fluid had a pH of 7.8. In it a normal muscle was much less ductile than when exposed to the air and gave a permanent deformation of only 1.41 mm. A muscle, immersed in a liter of this same standard Ringer to which there had been added 6 cc. .012 N HCl, bringing the pH to 7.6, was even less ductile than that stretched in fluid with a pH of 7.8. The addition of 16 cc. of .012 N HCl to 1000 cc. of standard Ringer gave a fluid with a pH of approximately 7.3; and in this a muscle stretched very much as

when exposed to the air, giving a permanent deformation of 3.1 mm. The addition of 19 cc. of .012 N HCl to 1000 cc. of standard Ringer gave a pH of approximately 7.2 and in this a muscle stretched more than when exposed to the air, giving a permanent deformation of 5.8 mm.

CHART I.

Variations in the ductility of muscle, expressed in mm. of permanent elongation caused by stretching. Since stress and time were the same in all experiments, the differences shown here were due to differences in the condition of the muscles.



It is, therefore, apparent that the ductility of muscle is influenced by relatively slight changes in the hydrogen ion concentration of the fluid with which it is bathed. It is obvious that these observations may have an important bearing on the problem of tonus and the function of muscles as organs for the maintenance of posture.

3759

The Lactic Acid Content of Resting Mammalian Muscle.

H. A. DAVENPORT AND HELEN K. DAVENPORT
(Introduced by P. A. Shaffer.)

From the Department of Neuroanatomy, Washington University Medical School.

The amount of lactic acid present in resting, atonic mammalian muscle is very low—10 to 20 mg. per 100 gm. of tissue. We have obtained values from 10.5 to 50 mg. by freezing *in situ* the gastrocnemius of guinea pigs with CO_2 snow. The animals were anesthetized with amytal and the muscles dissected as free from surrounding structures as possible without disturbing the nerve and

blood supply. Packing CO_2 snow around the muscle, starting at the insertion and proceeding to the origin, has given the lowest values for lactic acid.

Fletcher[1] found 59 mg. per cent in rabbit muscle, but excised the muscle before freezing it in liquid air. We have obtained similar results from animals which died (from the anesthetic) during the dissection, even though the muscles were frozen *in situ* immediately and without stimulation. It seems likely that mammalian muscle forms lactic acid with such rapidity when oxygenation is stopped that it is possible to obtain a value approximating the normal only when it is frozen with the circulation still intact.

We have used ice cold trichloracetic acid (5% aqueous solution) as a protein precipitant. After freezing, the muscles were excised and the unfrozen portion at the origin discarded. They were then sliced, while frozen, into sections of about 0.1 mm. thickness and immediately dropped into the trichloracetic acid. Lactic acid was determined by the Friedemann, Cotonio and Shaffer[2] procedure adapted to quantities between 0.02 and 0.2 mg.

[1] Fletcher, W. M., *J. Physiol.*, 1914, xlvii, 361.

[2] Friedemann, T. E., Cotonio, M., and Shaffer, P. A., *J. Biol. Chem.*, 1927, lxxiii, 335.

3760

Inhibition of Lactic Acid Formation in Muscle by Extract of Desiccated Pancreas.

E. RONZONI. (Introduced by D. P. Barr.)

From the Department of Internal Medicine, Washington University School of Medicine.

Hopkins and Winfield[1] showed that extracts of pancreas could inhibit the formation of lactic acid in chopped muscle. This work did not exclude the possibility that the inhibitory factor was trypsin. Foster and Woodrow[2] working in Hopkins' laboratory made further investigation of this substance. They found that it was neither trypsin, insulin nor anti-gloxylase, and that heating it for 10 minutes at 85° C. greatly reduced its activity but did not completely destroy it. They reported an inhibition between 40 and 60% of the total lactic acid which was formed in control samples of muscles, a fact which they interpreted as evidence of two mechanisms for lactic

acid production in muscle, one of which was inhibited by the pancreatic factor.

In an attempt to determine the mechanism of the action of this substance, we confirm the observations of Foster and Woodrow,[2] obtaining an inhibition of between 40 and 60% of the total lactic acid production in muscle hash in concentrations similar to theirs (extract of 5 mg. of desiccated pancreas acting on between 3 and 4 gm. of muscle in 10 cc. of phosphate buffer). Increasing the concentration produces no greater inhibition. This action is not due to trypsin as Foster has demonstrated. That the inhibiting factor, as prepared, usually contains no trypsin is shown by the fact that filtrates after $HgCl_2$ precipitation of the proteins give no Biuret test. The non-protein nitrogen content of muscle extracts treated for 3 hours with the inhibiting factor usually shows no increase. In a few cases, there occurs a rise from 1 to 2 mg. percent, an increase which does not affect the inhibitory action of the extract upon lactic acid formation.

A change in pH or in salt concentration is not the cause of the inhibition. Certain inactive extracts having the same salt concentration and pH as active extracts have had no inhibiting effect. In all experiments which are reported here the pH has been carefully controlled by regulating the pancreatic extract to the pH of the buffer before adding it to the reaction mixture. It has been shown that the inhibition is not due to direct action of the extract upon the carbohydrate itself. That the inhibiting factor as prepared is rich in amylase is shown by its action on glycogen solutions. In a period of 2 hours, this extract acting on a 0.5% glycogen solution, in a concentration similar to that used in the experiments, converts from 50 to 60% of the glycogen into a free reducing substance. However, some pancreatic preparations showing a marked amylolytic activity have been inactive as inhibitors of lactic acid formation. In hashed muscle, the inhibiting factor not only prevents the formation of lactic acid but also interferes with the disappearance of carbohydrates. The inorganic phosphates, on the other hand, are increased to the same extent as when the inhibiting factor is not employed.

If the increase in free phosphate may be taken as a measure of the breakdown of hexose phosphate and if the presence of hexose mono- and diphosphate may be assumed, the changes in phosphate will account for the lactic acid which is formed. In muscle, therefore, it appears that the formation of lactic acid from preformed hexose phosphate is not inhibited by the pancreatic extract.

TABLE I.

The influence of the inhibiting factor on changes occurring in hashed muscle.
Expressed as mg. per 100 gm. of muscle.

Source of muscle	Lactic Acid Increase		Phosphate Increase expressed as glucose from hexose monophosphate		Total Carbohydrate Loss expressed as glucose	
	Control	Inhibitor	Control	Inhibitor	Control	Inhibitor
Rabbit	842	420	480	496	430	20
Rabbit	647	342	378	393	315	13
Rabbit	728	389	420	430	340	00
Chicken	467	238	252	268	220	16

Upon the muscle extract of Meyerhof[3] the inhibiting substance has a similar but more striking effect. It almost completely prevents the formation of lactic acid. It allows of no loss in the total carbohydrate. Its effect upon free phosphate concentration, however, differs from that observed in hashed muscle. In Table II it will be seen that there is no change in free phosphate concentration in the experiments where the inhibitor has been added.

TABLE II.

Influence of the inhibiting factor on changes occurring in muscle extract.
Expressed as mg. per 100 cc. of muscle extract.

No.	Lactic Acid Increase		Phosphate Changes expressed as glucose from hexose monophosphate		Total Carbohydrate Changes expressed as glucose	
	Control	Inhibitor	Control	Inhibitor	Control	Inhibitor
1	+ 93.6	+2.3	—13.6	+2.60	—100.0	±0.0
2	+106.0	+0.6	—12.9	+1.8	—113.0	—0.3
3	+101.0	+0.9	—15.2	+1.2	—110.0	—1.1
4	+ 68.0	±0.0	—23.2	±0.0	— 95.0	—0.4

The inhibiting factor prevents the loss of phosphate in muscle extract. Since the same extract shows no loss in carbohydrate, we must assume that the formation of hexose phosphate has been inhibited and that the point of action of the inhibiting substance is on the formation of hexose phosphate. Since there is no preformed hexose phosphate in muscle extract and its formation can be inhibited by the pancreatic factor, complete checking of carbohydrate loss and of lactic acid formation is possible.

[1] Hopkins, F. G., and Winfield, G., *J. Physiol.*, 1915, l, 5.

[2] Foster, F. L., and Woodrow, C. E., *Biochem. J.*, 1924, xviii, 562.

[3] Meyerhof, Otto, *Biochem. Z.*, 1927, clxxxiii, 176.

3761

Experimental Production and Prevention of Acute Edema of the Lungs in Rabbits.

SCOTT JOHNSON. (Introduced by Leo Loeb.)

From the Department of Pathology, Washington University School of Medicine.

It is a common occurrence that the intravenous injection of large doses of adrenalin hydrochloride into rabbits causes the development of acute edema of the lungs and death shortly afterwards. It was found that injection of 1 cc. of 1/1000 solution of adrenalin hydrochloride (Parke, Davis & Co.) produces acute edema of the lungs in almost all medium sized rabbits.

While studying the effect of the action of adrenalin on the heart in the production of experimental myocarditis according to the method of Fleisher and Loeb,[1] we found that if the thorax of the animal is opened so that the heart and lungs are exposed to the air, and if then 1 cc. of adrenalin is administered intravenously, acute edema of the lungs does not occur. We also observed that acute edema of the lungs, which ordinarily would follow the injection of 1 cc. of adrenalin, could be prevented through the opening of the thorax by means of a cut made into the sternum in the midline and the subsequent opening of the pericardium, leaving at the same time the pleural sacs intact. This experiment was repeated several times with the same result. This observation was made independently by Dr. Leo Loeb[2] in 1909, but was never reported.

It occurred to us that the only factor that had been altered by opening the chest was the difference in pressure between the external air and the air in the lungs. We, therefore, attempted another experiment to confirm our previous observations. The rabbit was tracheotomized and a cannula was inserted tightly in the trachea. Air from an artificial respiration apparatus was allowed to pass into the lungs at a pressure greater than that of the atmosphere, the chest remaining intact. One cc. of a 1/1000 solution of adrenalin hydrochloride was then injected intravenously into the rabbit and acute edema of the lungs did not occur. The experiment has been repeated with the same result. In reviewing the literature we found that Haven Emerson[3] had made the latter observation in 1909.

We were able to observe directly the mechanism through which the pulmonary edema is produced, by watching the exposed heart after the administration of adrenalin in large doses. We thus

found that as a result of the peripheral vaso-constriction of the arterioles, and the subsequent rise in systemic blood pressure, the left ventricle attempts to compensate for this impediment to the flow of the blood by the increased force of its contractions. In several instances we have seen the left ventricle go into its systolic position and relax only very slightly in diastole while the right ventricle appears to contract more vigorously than normally. The result of these changes is an obstruction to the circulation of the blood on the left side of the heart, but not on the right. This eventually leads to an over-distention of the capillaries of the lungs with blood. If the capillaries distend widely enough the blood fluids escape through the capillary walls into the alveoli of the lung and edema is thus established. If the pressure on the capillary wall of the lungs and the subsequent distension becomes still greater, the red corpuscles may escape together with the fluids into the tissue spaces and alveoli.

It should be possible to prevent this type of edema by preventing the undue dilatation of the lung capillaries. Either through opening the chest or through inflating the lung under increased pressure with the chest closed, can this condition be fulfilled. A quantitative relation, undoubtedly, exists normally between the pressure of the air in the alveoli acting as a support of the capillaries from the outside, and the pressure of the blood inside the capillaries which depends directly upon the force of the contraction of the right ventricle. When pressure conditions are changed by obstruction in the left heart, or by increased strength of the beat of the right heart, or by both these factors combined, the capillaries of the lung become distended with blood, and if distended widely enough they leak. However, if the capillaries are prevented from over-dilating by increased air pressure acting on them from the interior of the lung, they do not leak and edema of the lungs is therefore prevented by these means.

Chillingsworth and Hopkins[4] were able to reduce the carotid pressure to zero by reducing the air pressure on the exterior of the body in dogs which had been placed in a plethysmograph, but the lungs of which were left in communication with the outside. It appears to us that acute edema of the lungs in rabbits due to the administration of large doses of adrenalin should also be preventable by the application of a similar method and we intend to test this suggestion in the near future experimentally.

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[1] Fleisher, M. S., and Loeb, Leo, *Arch. of Int. Med.*, 1909.

[2] Loeb, Leo, personal communication.

[3] Emerson, Haven, *Arch. of Int. Med.*, 1909, iii, 68.

[4] Chillingsworth and Hopkins, *Am. J. Physiol.*, 1920, li, 289.

3762

On Ovarian Regeneration in the Albino Rat.

FRANK BLAIR HANSON AND FLORENCE HEYS.

From the Zoological Laboratory, Washington University.

Numerous cytological papers on germ cell origin have led to the view that the primordial germ cells are not the ancestors of the functional germ cells, although work is not lacking in which the older view is upheld that germ cells form a continuous stream throughout the individual life cycle and from generation to generation.

Davenport,[1] spaying mice, found that, after a lapse of from 8 to 45 weeks, regeneration of the ovary occurred in 64% of his animals. Complete removal of the ovary was claimed, but cytological evidence was not secured in support of this. Macroscopic examination of masses of tissue at or near the site of the operation was the criterion for regeneration of the ovary. Haterius[2] secured 4 cases of regenerated ovarian tissue in mice out of 76 operations, and attributed these to incomplete ovarian extirpation. Parkes, Fielding and Brambell[3] had 121 cases of double ovariotomized mice. After ovariotomy the oestrous cycle (vaginal smear method) ceased. But in 11 cases spontaneous oestrus subsequently occurred, and this was taken to indicate regenerated ovarian tissue. In 8 of these 11 cases the presence of new ovarian tissue was demonstrated histologically.

We are able to add data of a similar nature from the albino rat. Both ovaries were removed from 105 rats and each ovary preserved for sectioning. The animals ranged in age from 10 to 200 days at time of operation. The period allowed for regeneration ranged from 90 to 180 days. There were 8 cases of ovarian regeneration as determined by sectioning the regenerated masses of tissue. The 8 original ovaries removed from these sites of regeneration were then sectioned to determine whether the whole ovary had been removed.

In 2 of the 8 original ovaries incomplete removal was demonstrated cytologically, while in 6 cases extirpation was apparently complete. This gives a regeneration rate of 3.5% in the rat. In the mouse Davenport got 64%; Haterius a little over 5%; and Parkes, Fielding and Brambell 15%.

Our work suggests that the younger the animal when spayed the less likelihood of regeneration. None of our rats under 40 days of age showed regeneration, although rats from 10 to 40 days of age

at time of operation were allowed the longer period (180 days) in which to regenerate. Success in complete removal of the ovary increases as the age at which animals are spayed decreases. This fact leads us to believe that the last word has not yet been said on this subject of mammalian ovarian regeneration, and a further series of experiments based exclusively on very young rats has been initiated.

[1] Davenport, C. B., *J. Exp. Zool.*, 1925, xlii, 1-11.

[2] Haterius, H. O., PROC. SOC. EXP. BIOL. AND MED., 1927, xxiv, 784-786.

[3] Parkes, A. S., Fielding, U., and Brambell, F. W. R., *Proc. Roy. Soc.*, Series B, 1927, ci, 328-354.

3763

Regurgitation of Duodenal Contents as Factor in Neutralization of Gastric Acidity.

ISAAC Y. OLCH AND ROBERT ELMAN. (Introduced by E. A. Graham.)

From the Department of Surgery, Washington University School of Medicine.

Considerable work has been done on gastric function, but this has been mainly concerned with the motor activity of the stomach. Comparatively little has been done on the chemical activity, that is, the method of secretion, the nature of the secretory stimulus, and the variations of the reactions of the gastric contents. We have lacked exact methods for investigating these functions. In the past, the test meals, of many kinds, were used. Inasmuch as results with any one type of test meal vary greatly among individuals who are apparently normal, and also vary so much among patients afflicted with a given type of gastric disorder, the uselessness of this mode of examination is evident. A more exact method has been described by Bloomfield and Keefer,[1] who stimulated gastric acidity with 50 cc. of 7% alcohol to which a small amount of phenolphthalein had previously been added. Here, again, is a method in which an attempt is made to gauge the chemical activity by the amount of free HCl secreted. We know that there are many individuals, free from any gastric symptoms, in whom the HCl content of the stomach varies from a hypochlorhydria to an achlorhydria; on the other hand, most individuals with hyperchlorhydria give symptoms which are referrable to this condition. It seemed to us, then, that conditions such as these in the stomach, were not due to secretory defects alone, but to a combination of secretory and motor

activity. The question then arose, how to measure these, simply and accurately.

In 1914, Boldyreff[2] introduced a certain amount of 0.5% HCl into the stomachs of dogs. This is the concentration at which HCl is normally secreted in the stomach. In the course of an hour or more, the acidity of the fluid decreased and it left the stomach. He thought that this neutralization was due to regurgitation of duodenal contents into the stomach. The strongly alkaline constituent here is pancreatic juice, which, we know, is secreted due to the presence of acid in the stomach. The bile and intestinal juices play minor rôles. Boldyreff proved this by tying off the pancreas and then found that neutralization did not occur. Elman[3] also has shown this to be the case in his animals with pancreatic fistulae. Hawk[4] and his associates have demonstrated this regurgitation of pancreatic juice in the resting stomach by the presence of trypsin in the gastric juice.

We repeated these experiments, introducing 200 cc. of 0.5% HCl into the stomach of an apparently normal dog. Ten cc. were withdrawn for examination every 10 minutes. We found that in the normal animals the titration at any given interval was practically the same. By averaging the results in many dogs we were able to construct a normal curve. Starting with a titration of 140 free HCl (0.5%) the decrease is gradual down to 49 free HCl at 90 minutes. The stomach usually emptied in from 90 minutes to 2 hours. In some of the experiments the fluid in the stomach was withdrawn at the end of each 10 minute period, measured and reintroduced. In many instances the amount withdrawn at the end of a 10 minute period was found to be greater than the amount introduced into the stomach at the beginning of the period. This increase, since it was accompanied by a drop in acidity, was due to the alkaline duodenal contents which had poured back into the stomach. If this increase were secreted gastric juice, there would have been no decrease in acidity. The exact mechanism of this phenomenon is as follows: Acid gastric juice, especially at the concentration at which it is secreted, will not be tolerated by the sensitive duodenal mucosa. Instead, large amounts of alkaline pancreatic juice are secreted and this fluid pours back into the stomach to decrease the gastric acidity to that point at which the duodenum will accept it. The reaction of the gastric contents, then, at any given time in the course of digestion is the resultant of secretion and neutralization by regurgitation. The prepylorus, pylorus and duodenum may well be regarded as a singie organ, a mixing chamber where the acid material is prepared for the intestines and it is in

this region that most of the gastro-duodenal lesions occur. We propose the above described method of examination, because it gives us a more or less exact measure of the physiologic activity of this mixing chamber. Unlike most methods of gastric study, we are here dealing with known amounts of known substances.

We next tried the effect of certain operative procedures upon the stomach. First we cut the vagi, either intrathoracically or intraperitoneally, or we severed the intrinsic nerve supply by cutting around the stomach down to the mucosa. Examination of these animals later showed a decreased emptying time, which is what Hughson found in studying the motor function after vagotomy. There was also a more rapid neutralization. This is due to the fact that without vagus control the pylorus loses its tone, is more patulous and allows quicker neutralization. The latter also explains the decreased emptying time.

We next subjected normal animals to either gastroenterostomy, Polya-Balfour resection, or pyloroplasty. In all these instances there is decreased emptying and quicker neutralization, and this is particularly the case following pyloroplasty or resection. The neutralization in the gastro-enterostomy was about the same as after vagotomy.

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[1] Bloomfield and Keefer, *Arch. Int. Med.*, 1926, xxxviii, 141.

[2] Boldyreff, *Quar. J. Exp. Physiol.*, 1914, vii, 1-12.

[3] Elman, Robt., to be reported.

[4] Hawk, *Am. J. Physiol.*, 1916, xxxix, 459.

3764

Decerebrate Pigeon-Fear Signs

ALFRED OGLE SHAKLEE.

From the Laboratory of Pharmacology of St. Louis University School of Medicine, St. Louis, Mo.

Based on the statements of Flourens,[1] McKendrick,[2] Ferrier,[3] Vulpian,[4] Schrader[5] and others,[6] it is still believed that the pigeon deprived of its cerebral hemispheres, "exhibits no signs of fear." In the study of such a pigeon,[7] I observed repeatedly, indubitable signs of fear. Some of these signs were: dodging, starting, trembling, fleeing, struggling, staring, and crying out in a characteristic way. Since these signs, which show the semblance of fear, were produced

in this decerebrate pigeon by stimuli that ordinarily produce fear in nomal pigeons, they may, perhaps, be called signs of fear.

As examples of fear reactions we cite only a few, as follows:

Sound Fear Reactions—One hour after the operation, clapping of the hands caused the head to move, the body to tremble, and the wings to shuffle. Fifteen hours after the operation, clapping the hands caused the head to rise and the eyes to stare. On the 42nd day after the operation, snapping a ruler on a table 8 to 10 times in succession, caused the head and body to turn toward the sound, the neck to crane, and the eyes to stare. On the 5th day after the operation, the clock struck one out in the hall, about 30 feet away, the neck craned, the head turned toward the sound, and the eyes stared. On the 40th day, the noise and talking of the ice boys out in the hall, about 30 feet away, caused movements similar to those just mentioned, resulting in the attitude and expression of fear.

Reactions to Grasping and Sight(?)—Eleven days after the operation, the pigeon was grasped gently with both hands and tilted toward some water. This caused considerable struggling. On the 13th day, when the pigeon was lightly grasped, it struggled, with signs of slight fear, and tried to escape; grasping a leg caused vigorous struggling with leg and wings, and signs of fear. On the 43rd day, grasping a wing, lightly, caused hard struggling, signs of fear and fear sounds. On the 47th day grasping either wing or leg gave a reaction like that just mentioned, giving the semblance of fear.

Sight Reactions—On the 4th day, the rapid approach of a hand caused the eyes to close and the head to jerk. On the 9th day, a slow approach of the hand caused the eyes to stare with the semblance of fear. A brusque approach of both hands caused a quick, slight start of the body. This same stimulus, on the 18th day, repeatedly caused the eyes to close and both the head and body to jerk (dodging). On the 37th day, this stimulus caused a quick retreat of 4 or 5 steps. On the 40th day, a slow movement of the hand close to the head caused dodging. On the same day, reaching gently to take him up caused him to retreat with uneasy expression. On the 49th day, this caused fear sounds and actions.

Reactions to Pursuit—Sight and Sound(?)—On the 34th day, slow, close pursuit caused a retreat of 4 to 5 steps with attitude and expression of alarm. On the 40th day, rapid pursuit caused actual running away, with the utterance of low-pitched fear sounds. This was repeated several times on the succeeding days. On the 48th day, 3 or 4 times in succession, he fled, running, with head turned to the side, watching me; and one time, with flapping of the wings.

The pigeon was number 3 of the series reported by me in 1921.[7] It was a full grown pigeon, recovered quickly from the operation, and remained healthy and in good condition throughout the 49 days of study.

A microscopic study of the brain by means of serial sections prepared by Mr. Martin W. Schmidt, using the Krause method, was made by Dr. Albert Kuntz. A small residue of apparently living cerebrum was found distributed in three different regions. Two of the regions were contralateral duplicates of each other, located on the mid-ventral surface of the cerebrum, just anterior to the anterior plane of the optic chiasma; and each averaged roughly 1.5 mm. wide, 2.5 mm. high, and 2 mm. long. These regions revealed some good cerebral tissue, with some medullated fibers and neuroglia, apparently in good condition. They also showed in parts, evidences of degeneration and infiltration. The third region was roughly globular in shape, about 1.5 mm. in diameter, and, cut by the frontal plane of the optic chiasma. It lay near the ventral surface of the cerebrum, about 4 mm. from the sagital fissure. In it there was evidence of degeneration. There were no medullated fibers present. "Holes" showed where cells had been absorbed, and the cells remaining were scattered. The brain stem showed a large blood clot in its center with necrotic tissue around it.

[1] Flourens, P., *Recherches expérimentales sur les propriétés et les fonctions du system nerveux,* Paris, 1842.

[2] McKendrick, F. J., *Recent Researches on the Nervous System,* Edinburgh, 1874.

[3] Ferrier, David, *The Functions of the Brain,* London, 1876.

[4] Vulpian, E. F. A., *Leçons sur la physiol. du système nerveux,* Paris, 1866.

[5] Schrader, M. E. G., *Pflüger's Arch. f. ges. Physiol.,* 1889.

[6] Howell, *Textbook of Physiology,* Sept., 1927.

[7] Shaklee, A. O., *Am. J. Physiol.,* 1921, lv, 65.

3765

The Thyroid in Infections and Toxemias.

W. H. COLE AND N. A. WOMACK. (Introduced by E. A. Graham.)

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The work of Baumann,[1] who demonstrated in 1895 that the thyroid gland contained iodine, and of Oswald,[2] who a few years later isolated thyro-globulin and the nucleoproteins, contained the

basic facts upon which the modern advancements in prevention and treatment of goiter have been dependent.

We have observed, as has been noted by others, that the normal histological picture in animals, especially the dog, is somewhat variable. In a series of experiments upon dogs, we undertook to produce pathological changes in the thyroid and noted marked changes, many of which were of the hyperplastic type, after production of certain kinds of infections and toxemias. The thyroid glands of laboratory animals which succumbed to peritonitis, pneumonia, etc., most of which were secondary to an operation, were removed and analyzed for iodine content after a small piece was removed for microscopical section. Careful weights of the glands were obtained, making deductions for the pieces removed for section, and iodine determinations made according to the method of Hunter,[3] as modified by Kendall.[4] Peritonitis was also produced by ligation of the appendix and its mesentery, and empyemata produced by injection of streptococci and staphylococci into the pleural cavity. Sections of the thyroid were also removed from animals suffering from intestinal obstruction and severe subcutaneous infections produced by the injection of fecal material. In practically all animals, which were killed by either of the methods mentioned above, the iodine content of the thyroid gland was found to be diminished, and a quite constant destruction of acinal cells noted, with an accompanying attempt at hyperplasia.

In 7 dogs which were killed by the methods mentioned above, the iodine content was compared to that of 7 control dogs of equal weight which were kept and fed under the same circumstances as the dogs which died from infection produced by operations, etc. Analyses revealed 0.147 mg. iodine per kilo of body weight in the thyroids of the dogs with infection and 0.382 mg. per kilo body weight in the thyroids of the healthy control dogs. In other words, the thyroid gland of the healthy animals contained slightly more than twice the amount of iodine which was contained in dogs with infection. The weights of the thyroids, however, varied in a reverse manner. There was 0.294 gm. thyroid tissue per kilo of body weight in the dogs with infection, whereas the healthy control animals had only 0.191 gm. thyroid tissue per kilo of body weight.

There is a gross relation of the amount of colloid to the amount of iodine found by analysis, but instances were encountered when this relation was by no means exact. Many of the thyroids in the dogs which succumbed to infection were so cellular as to be absolutely free from any visible colloid, but were found to have 50 to 60% as much iodine as the thyroid in a normal dog of equal weight.

Apparently, the thyroid cells contain a substance which holds iodine in chemical combination within the cell.

The increase in size seems best accounted for by a hyperplasia. One might expect edema to be an important factor, but, as a matter of fact, very little evidence of edema is seen in the microscopical sections. The microscopical picture of the glands, removed from the animals with infections, varies. There are certain characteristics, however, which are quite constant. A desquamation of the acinal cells into the alveoli with disintegration of the protoplasm, is a constant finding. The cells remaining intact loose their cuboidal shape and become columnar in type. In many cases there is a heaping of acinal cells with papillary ingrowths into the alveoli, in a manner which can scarcely be differentiated from an exophthalmic goiter as found in human beings. Loss of colloid is also universal. This occurs in certain areas, about the section, and resembles the distribution of colloid as seen in a thyroid gland of a patient with a toxic goiter, to whom iodine has been given. In the latter case, however, the colloid is reappearing instead of disappearing.

In a series of thyroids removed at autopsy by Dr. S. M. Gray, a large percentage were decidedly abnormal. These abnormal glands were usually encountered in patients who succumbed to infections. They revealed the pathological changes, including desquamation, hyperplasia, loss of colloid and increased scar formation, which are seen in animals. The thyroid of one patient presented areas which were characteristic of early changes as seen in exophthalmic goiter, adenoma, and thyroiditis.

A lack of iodine in the food or drinking water had previously been assigned as a cause of epidemics of goiter. However, McCarrison[5] cites an instance, with other data, where the cause and control of endemic goiter had no relation to the amount of iodine in the drinking water. The discovery by Oswald,[6] that the thyroid gland of a patient with exophthalmic goiter contains less iodine per gram of weight than does the normal gland is pointed evidence that there is a relation between iodine metabolism, and toxic goiter. Marine and Lenhart[7] claimed a constant relationship between the iodine content and the structure of the gland. The fact, however, that the total amount of iodine in a normal gland and one from a patient suffering from exophthalmic goiter is about equal, suggests that there are other factors, probably more important than the iodine metabolism or deficiency in the production of goiter.

An inconstant swelling of the neck during the course of certain infectious diseases including measles, scarlet fever, typhoid fever, etc., has been recognized for years and emphasized by Garnier.[8]

Roger and Garnier,[9] as well as McCarrison[10] have reported pathological changes in animals following certain infections and toxemias. McCarrison[11] has recently observed that the histological structure of a normal thyroid gland (of rats) can be altered by feeding unbalanced diets.

Summary. All of the data presented in this article point strongly to the fact that the thyroid gland takes an active part in the mechanisms combatting diseases of the body in general. Especially does this seem true in acute infections and fevers. This active relation is not surprising, when we consider the part played by the thyroid in the metabolism of the body. Lesions in the thyroid have been produced by infections and toxic means which strongly resemble the microscopical pictures seen in exophthalmic goiter and toxic adenoma as seen in the human. Since the iodine content of the gland is reduced so markedly during acute infectious processes, experimentally, it seems logical to assume that administration of iodine to patients with infectious processes, especially of the acute type, might be beneficial. We are not yet prepared to state what the action of iodine in such cases might be, and we, therefore, are in no position either to advocate it or to state what might be considered safe dosages in such conditions.

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[1] Baumann, E., *Z. für physiol. Chemie,* 1896, xxi, 318 and 481; 1896, xxii, 1.

[2] Oswald, A., Strassburg, 1900; *Princip. Biochem. Centralb.,* 1902-1903, i.

[3] Hunter, A., *J. Biol. Chem.,* 1910, vii, 321.

[4] Kendall, E. C., *J. Biol. Chem.,* 1914, xix, 250.

[5] McCarrison, R., *Brit. Med. J.,* 1927, i, 94.

[6] Oswald, A., *Virchow's Arch.,* 1902, clix, 444.

[7] Marine, D., and Lenhart, C. H., *Arch. Int. Med.,* 1909, iv, 440. *Ibid.,* 1911, vii, 506.

[8] Garnier, M., *Le glande thyroide dans les maladies infectiouses.* Paris, 1899.

[9] Roger, H., and Garnier, M., *Compt. rend, Soc. de biol.,* 1898, l, 890. *Presse Medicale,* Paris, 1899, i, 181.

[10] McCarrison, R., *Lancet,* 1927, i, 916.

[11] McCarrison, R., The Thyroid Gland. London, 1917.

Peking Branch.

Peking Union Medical College, November 3, 1927.

3766

Migration Route of Spirocerca Sanguinolenta in its Definitive Host.

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Five years ago the writer found larval nematodes encysted on the mesentery of the hedgehog, *Erinaceus dealbatus,* secured in the vicinity of Peking. On morphological grounds Schwartz[1] determined these larvae to be *Spirocerca sanguinolenta.* Subsequent examination of specimens of this host revealed the fact that it was commonly infected with these larvae, encysted in the mesentery, in the omentum, and on the peritoneal wall of the stomach. Since living material was readily obtained during the entire year, an opportunity was offered to further study this problem. The dogs and cats used in the experimental series were born and reared in the laboratory. Although the cat is not a natural host of the infection, it has been found to be an appropriate one for experimental work and has proved to be as satisfactory in all respects as the dog.†

Mesentery and omentum containing the encapsulated larvae were fed to the animals immediately after the removal of these tissues from anesthetized hedgehogs. A half hour to an hour after feeding, the experimental host invariably attempted to vomit the meal. In the vomitus were active excysted and excysting larvae, the delicate adventitious capsule having been digested off almost immediately after coming in contact with the gastric juice. The vomitus was refed, the mouth was tied and digestion proceeded during forced retention. The animals were autopsied from 2 hours to 6 months after the feeding as indicated in Table I. After exposing the abdominal and thoracic viscera from its ventral aspect, the stomach was tied off from the esophagus and the duodenum, and the following vessels clamped:

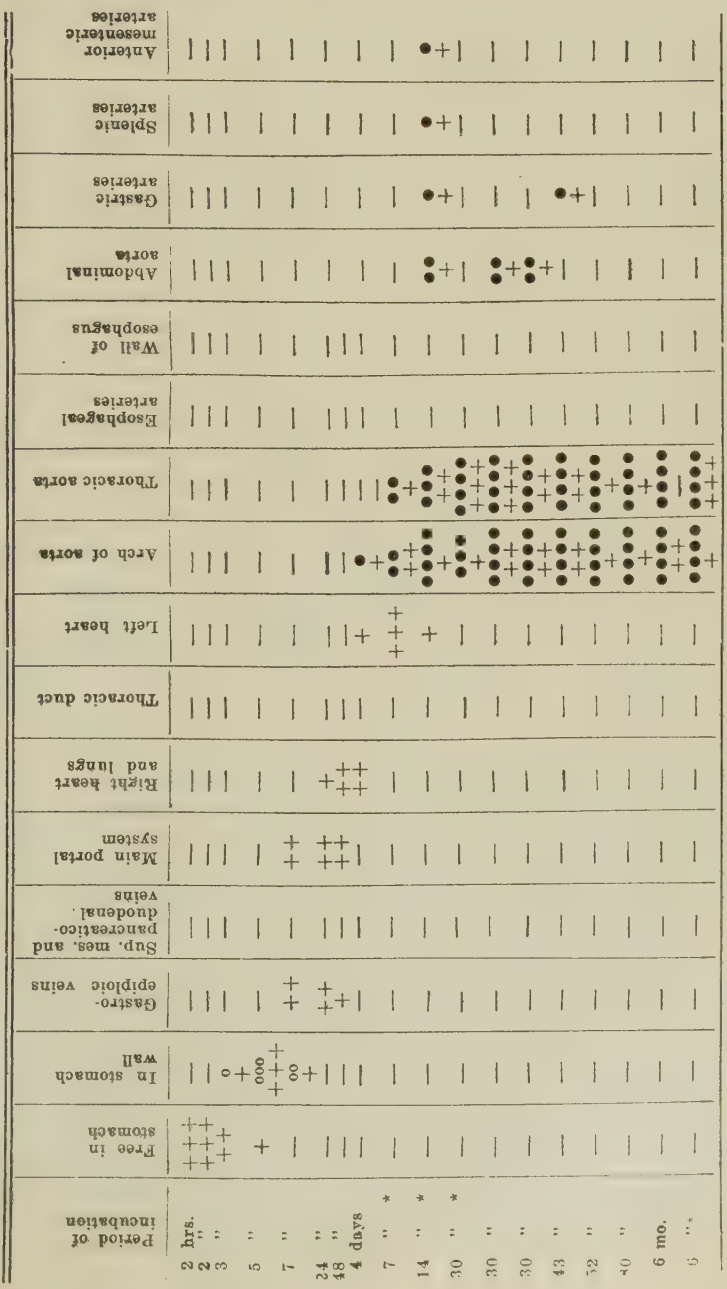
* Contribution No. 90.

† Rabbits have been found to be inappropriate hosts.

the mesenteric and splenic veins below the gastro-epiploic arteries, the coronary vein, the portal vein just below the liver, the inferior *vena cava,* the thoracic duct just below its opening into the left

TABLE I.

Relative number of Spirocerca worms recovered and of lesions produced during successive stages of migration from the stomach to the arterial circulation.



subclavian vein, and the pulmonary veins. The entire viscera were then removed, the several portions of the venous circulation separated from one another, and immediately irrigated with citrated saline. The stomach and esophagus were then opened along the midventral aspect, contents removed, and the walls carefully searched for lesions or larvae.

The lungs, heart, and aorta with adjacent arteries were then examined for lesions or worms. Within the period of observation, worms were recovered successively from the lumen of the stomach (2-5 hrs.), the stomach wall (3-7 hrs.), the gastro-epiploic veins (7-48 hrs.), the main portal veins (7-48 hrs.), the right heart and lungs (24 hrs. to 4 days), the left heart (7-14 days), the arch of the aorta (4 days to 6 months), the thoracic aorta (7 days to 6 months), the abdominal aorta (14-30 days), the gastric arteries (14-43 days), and the splenic and anterior mesenteric arteries (14 days). Although no worms were recovered from the carotid or subclavian arteries, in four kittens and two dogs paralysis of the hind legs,‡ beginning about the fifth day and continuing until autopsy, suggested that the spinal arteries had been blocked by larvae. Petechial lesions were prominent in the stomach wall from the second to the seventh hour after feeding (*i. e.*, during the period of migration of the larvae through the wall) but disappeared soon afterward. Lesions were commonly found in the arterial system wherever worms had lodged and penetrated. In no other part of the viscera were *Spirocerca* larvae ever recovered, nor were lesions ever found except those indicated.

Analysis of the data indicates that none of the larvae excyst in the esophagus and that not even the excysted larvae in the vomitus become attached to or penetrate the esophageal mucosa. The evidence accumulated in this experimental work is overwhelmingly opposed to the theory that the larvae penetrate directly from the lumen into the wall of the esophagus. At the same time, it constitutes positive proof that the route of migration through the stomach wall into the portal system and thence through the capillaries of the lungs into the arterial system is the usual one, and that the primary lesion is developed in the wall of the arterial system. The preponderance of lesions along the arch and the thoracic aorta in the experimental series, and in the corresponding region of the esophagus in natural infections suggests that some of the immature larvae migrate from the primary foci outward to the esophageal wall, finding there a favorable habitat in which to mature, mate,

‡ This condition was observed by Manson[2] in naturally infected dogs.

and produce embryonated eggs. These reach the lumen of the intestine only when an opening into the lumen is later developed. This is the only known means by which the eggs may reach the outside world and continue their development. This phase of the life cycle of *Spirocerca sanguinolenta* within its definite host is strikingly different from that of all other nematodes for which this part of the life cycle is known.

[1] Schwartz, B., *Proc. U. S. Nat. Museum*, 1926, lxviii, 5.
[2] Manson, P., *China Customs Med. Reports*, 1877, xiii, 24.

3767

Specific Dynamic Action of Food and Blood Coagulability.

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There occurs a marked shortening of the blood clotting time during the absorption of mixed meals. We have found this to be due only to the protein fraction of the foods, and to be intimately associated in time and degree with the specific dynamic action of this type of foodstuff.* Pure carbohydrate or fat meals produce little or no change in either the blood coagulability or basal metabolism. Glycocoll taken *per os* causes the same changes as does a protein meal. Strenuous exercise acts similarly.

The increased coagulability of the blood is evident in plasma divested of all platelets, but is more striking when platelets are present. We believe the change to be an increased reactivity of the clotting factors in both platelets and plasma, probably correlated with the greater cellular activity occurring throughout the body, which consititutes one part of the specific dynamic action of proteins.

The important clinical application of these facts lies in their relation to thrombosis—post operative, post partum, and following such inflammatory diseases as pneumonia, typhoid fever, etc. In all these conditions the tendency to thrombosis appears during convalescence, usually shortly after the patient has begun to partake of a full diet. Also during this convalescent stage the blood platelets are considerably increased in number, so that the coagulative phase

* Full details of the work will appear in the *Chinese Journal of Physiology.*

following protein intake would be even more striking than in normal individuals. The investigation is continuing along these lines.

3768

Peripheral Lesions Produced by L. Donovani and Allied Leishmaniae.

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Gonder,[1] Laveran,[2, 3] and Sergent[4] have reported peculiar lesions of the extremities, tail, scrotum, nose and ears in white mice inoculated intraperitoneally with recently isolated cultures of *L. tropica.* Sergent[4] was unable to produce such lesions with cultures beyond the 50th cultural passage.

Bramachari[5] and others in Calcutta have reported cases of Kala Azar treated with antimony and apparently cured, in whom there developed later, nodules in the skin of the face, upper extremities and trunk, in which leishmaniae were found and from which they were cultivated. The spleen, liver and blood stream in these cases were free from leishmaniae. Bramachari has called this complex "dermal leishmanoid". Acton and Knowles[6] reported a patient, diagnosed clinically as *xanthoma tuberosum multiplex.* A leishmania was cultivated from the lesions.

The present authors worked, in part, with cultures obtained from Drs. Nicolle and Anderson of Tunis, who have had them under continuous artificial cultivation for varying periods up to nearly 15 years. These cultures comprised *L. donovani (L. infantum),* strains KA and Sh, *L. canis* ("kala azar canin") strains x and X, and *L. tarentolae* ("leptomonas de gecko") from the gecko. All of these strains originally produced visceral lesions only. When these cultures were inoculated intraperitoneally into Chinese striped hamsters *(Cricetulus griseus),* the infections were visceral at first, with enlarged spleen and liver and with leishmania fairly abundant in the smears from the spleen, liver, bone marrow and heart blood. After a lapse of from two months to over a year from the time of inoculation, bilaterally symmetrical lesions began to appear, in the following order: (1) swellings of the carpi and tarsi extending later to the feet, including the digits; (2) swelling of the posterior half of

* Assisted by grants from the China Medical Board of the Rockefeller Foundation.

the scrotum in males, with subsequent ulceration, (infiltration and enlargement of the clitoris, exceptionally with ulceration of the perineum, in the female); (3) swelling (infiltration) and later ulceration of the base of the tail; (4) similar swelling of the nose, rarely with ulceration; and (5) swelling and ulceration of the margins of the ears. From the swollen tissues, leishmaniae enclosed in large mononuclear cells (clasmatocytes) were obtained, often in large numbers. The lesions of the feet never showed ulceration. The clasmatocytes were present often in enormous numbers between the fibers of the ligaments from the carpi and tarsi and distally (feet including digits). The clasmatocytes in the lesions showing ulceration, were in the deep layers of the skin and subcutaneous tissues. Intraperitoneal inoculation of the tissues from these peripheral lesions produced the same picture in 2 to 4 months and have continued to do so consistently and repeatedly. The same pathological changes were obtained with all the 5 strains used. As the peripheral lesions developed, those of the internal viscera tended to disappear, so that at autopsy some of the animals had normal-sized spleens and livers, negative for leishmaniae in smear. Intraperitoneal inoculation of such tissue did not cause infection in hamsters. Similar lesions of a single extremity were found in hamsters inoculated with two different Chinese strains of *L. donovani.* These hamsters, already showing heavy visceral infection, had been tied with leather thongs constricting the four extremities, during insect feeding experiments several months before the appearance of the lesions. Inoculation of tissue from these local swellings produced only general visceral infections like those caused by the strains with which these hamsters had been originally infected.

Inoculations of Acton and Knowles' "xanthoma" strain have produced only visceral lesions. The same is true of cultures from a case of "dermal leishmanoid". Cultures of both these strains were agglutinated by the sera of rabbits immunized against Indian strains of *L. donovani.* From these reactions it may be assumed that these organisms are *L. donovani.*

We acknowledge with thanks the courtesies of Drs. Nicolle and Anderson of Tunis, Dr. Knowles and Dr. Napier of Calcutta in supplying cultures.

[1] Gonder, *Arch. f. Sch. u. Trop. Hyg.*, 1913, xvii, 397.

[2] Laveran, *Comp. Rend. Acad. Sci.*, 1914, clix, 539.

[3] Laveran, *Bull. Soc. Path. Exot.*, 1915, viii, 363.

[4] Sergent, *Annales de l'Inst. Pasteur*, 1926, xl, 411.

[5] Bramachari, *Indian Medical Gazette*, 1822, lvii, 125.

[6] Acton and Knowles, Annual Report of the Calcutta School of Tropical Medicine, Calcutta, India, 1925.

3769

Action of Pseudo-Ephedrine on Uterus and Bladder.

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In a series of 90 experiments on isolated strips of rat, rabbit, guinea pig, dog and human uterus, and on muscle strips from the fundus and trigone of the rabbit bladder, suspended in 100 cc. Tyrode solution at 38° C., according to Burn and Dale's method, the characteristic and specific action of pseudo-ephedrine was sought. This was compared with the action of ephedrine, adrenaline and pituitrin.

Rabbit Uterus. Pseudo-ephedrine gives a prompt increase of tone, and rate of contractions. This accords with the results reported by Fujii[1] who concluded that the action in all doses is wholly on the muscle. It was found that, after ergotoxin paralyzes the augmentor fibers of the sympathetic, pseudo-ephedrine still causes contraction at the same or increased rate but with less increase in tone. Adrenalin, applied after the onset of these contractions, promptly obliterated them, probably a manifestation of the reversal effect of ergotoxine.

Pak and Read[2] have shown that pseudo-ephedrine increases blood pressure and has a diuretic effect, which add to the picture of a pituitrin-like action. This is supported, also, by the action, reported by Fujii[1] on the intestine, which responds to muscular stimulation by relatively large doses. The experiments here reported showed increase of tone and rate and a sustained effect in guinea pig and human uterus, the curves resembling those of pituitrin.

It was found that atropine has no effect on the action of pseudo-ephedrine in the rat uterus. The fundus of the rabbit bladder, with its parasympathetic innervation[3] is relaxed by atropin, but seems to be slightly stimulated by pseudo-ephedrine. The very slight effect on the fundus and absence of effect on the trigone, by pseudo-ephedrine, suggests a balanced muscle-nerve effect. More work is under way on this point.

This study will be continued on intact animals and, as opportunity offers, in clinical cases.

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[1] Fujii, M., *J. of Orient Med.*, 1925, iii, 1.

[2] Pak, C., and Read, B. E., *Chinese J. of Physiol.*, in press.

[3] Macht, D. I., *J. Pharm. and Exp. Therap.*, 1926, xxvii, 390.

3770

Effect of Denaturation on Digestibility of Ovalbumin by Pepsin and Trypsin.

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The object of this investigation is to throw light on the fundamental mechanism underlying the process of denaturation, by comparing the rates of digestion by pepsin and trypsin of natural ovalbumin with those of the same protein denatured in various ways. Increase in the digestibility of denatured proteins would point to hydrolysis or some other kind of degradation, while decrease in digestibility would indicate a change in the opposite direction.

Crystalline ovalbumin was prepared from egg white and, after dialysis, was used either as such, or after denaturation. The denaturing agents used were acid, alkali, heat, alcohol and shaking. The protein solution was mixed with a calculated amount of acid or alkali, enzyme solution and enough water to make a 1% of protein. A series of such mixtures of varying pH was prepared and maintained at 37.5° C. for a definite length of time, generally 4 hours, at the end of which the enzyme action was quickly stopped, by making the solution either acid or alkali as the case may be. The undigested protein was precipitated with trichloracetic acid and the soluble nitrogen in the filtrate was determined. After making corrections for the nitrogen derived from enzymes the percentage of total nitrogen digested was calculated. The optimal pH and the maximal digestion of the different proteins are shown in Tables I and II.

TABLE I.

Relative rates of peptic digestion of natural and denatured ovalbumin at their respective optimal pH's.

Time of incubation, 4 hours. Concentration of enzyme 0.2%.

Protein	Percentage digestion	Optimal pH
Natural	51	1.03
Alkali denatured	56	1.22
Acid denatured	52	1.37
Acid heated	52	1.63
Shaking-denatured	51.5	1.56
Alkali-heated	48	1.6
Alcohol denatured	37.5	1.4

TABLE II.

Relative rates of tryptic digestion of natural and denatured ovalbumin at their respective optimal pH's. Arranged in the order of increasing digestibility.

Protein	Concentration of enzyme	Duration of incubation	Percentage digestion	Optimal pH
	%	hour		
Natural	5	4	43	10.7
Acid-heated	5	4	46.5	9.25
Shaking-denatured	5	4	92	10.5
Alkali-heated	5	4	96	9.10
Acid denatured	5	1	96	10.30
Alcohol denatured	2	1	99	10.10
Alkali denatured	2	2/3	89	9.40

Summary: (1) In the peptic series the rates of digestion of natural and different forms of denatured proteins are, with a few exceptions, practically the same within the limit of experimental error. This indicates that the fundamental changes underlying denaturation do not affect those linkages in the albumin molecule which are hydrolyzed in peptic digestion. (2) In the tryptic series the rate of digestion of natural albumin is exceeded by all forms of denatured proteins. This finding indicates that the changes produced by the denaturing are in the direction of degradation and probably of the same nature as tryptic digestion. (3) Natural and denatured proteins have different optimal pH's of digestion. There is a tendency for these optimal points to shift toward the neutral point when the protein is denatured. Northrup[1] showed that the rate of digestion of a protein is, in general, a function of the concentration of ionized protein, and that it is minimal at the isoelectric point of the protein, and maximal at that pH at which the protein is completely combined with acid or alkali to form salts. In the light of this hypothesis our findings seem to indicate that the isoelectric point and the maximal dissociation of the albumin are shifted toward the neutral point by denaturation.

[1] Northrup, J. H., *J. Gen. Physiol.*, 1922, v, 263.

3771

Effect of Racemization on Digestibility of Casein and Egg Albumin by Pepsin and Trypsin.

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Racemized casein was prepared by Dakin and Dudley[1] by allowing N/2 sodium hydroxide to act on casein until the optical rotation fell to a minimum. Racemized egg albumin was prepared by Dakin[2] in a similar way. These authors claimed that neither racemized casein nor albumin was digested by proteolytic enzymes. In our preceding paper[3] we reported that ovalbumin denatured by dilute alkali was digestible by pepsin and trypsin. Since both racemization and denaturation are brought about by the action of alkali, the findings mentioned above are contradictory. A reinvestigation on the digestibility of racemized protein is, therefore, desirable.

Portions of racemized casein or egg albumin solution, after being adjusted to different pH's and mixed with pepsin or trypsin solutions, were incubated at 37.5° for varying lengths of time. At the end of the incubation period the undigested protein was precipitated with trichloracetic acid and the soluble nitrogen in the filtrate was determined. Our results showed that the digestion amounted to from 10 to 60% of the total N, even when corrected for complete autolysis of the protein contained in the enzyme preparation. We succeeded also in demonstrating that racemized egg albumin could be putrefied.

On the basis of these findings we conclude that racemized proteins are digestible by proteolytic enzymes.

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[2] Dakin, H. D., mentioned by Ten Broeck, C., *J. Biol. Chem.*, 1914, xvii, 369.

[3] Lin, K., Wu, H., and Chen, T., PROC. SOC. EXP. BIOL. AND MED., 1927, xxv, 199.

Minnesota Branch.

University of Minnesota Medical School, November 30, 1927.

3772

Resistance and Capacity of Stimulated and Unstimulated Muscle at Varying Electric Current Frequency Related to Chronaxie.

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The relation of chronaxie to the frequency of alternating electric currents capable of stimulating muscle is shown in the following table. The alternating current was derived from a General Radio Company oscillator, No. 377, and silver pin electrodes 5 mm. apart coated with AgCl by electrolyzing 1% NaCl with 6 volts for 10 seconds. These were stuck into the muscle and the chronaxie was determined by the condenser method.

TABLE I.

$\sigma = 0.001$ second. Kilocycle = 1000 cycles per second.

	Chronaxie σ	Duration of oscillator current in 1 direction σ	Maximum No. of kilocycles capable of stimulating
Fish	0.20	0.010	50
Frog	0.25	0.013	40
Crab	10.00	0.025	20
Scallop	30.00	0.050	10

The above table indicates that the threshold of duration of current from oscillator is about 0.05 of the chronaxie in case of fish and frog. With the crab and scallop the results are uncertain owing to the uncertainty of the determination of such long chronaxies by the condenser method. Since the energy required to stimulate is at a minimum at the chronaxie, it seems best to use muscles with short chronaxies for measuring the conductivity of the muscle with the stimulating current (using telephone for detection), so that the muscle will become stimulated in a small fraction of a cycle.

Lapique has shown that chronaxie varies rapidly after excision of tissue, and similar results are obtained for the highest frequency capable of stimulating the muscle. The gastrocnemius of the green frog was excised at 4:50 P. M. and immediately placed in moist chamber and stimulated at 70 kilocycles; at 4:55, 60 kilocycles was the highest frequency that would stimulate it; at 5:20, 30 K. C., 5:30, 28 K. C., and 5:45, 20 K. C.

No great difference in chronaxie was observed between bony fish and elasmobranch (dogfish) and the absence of bones in the latter made it convenient. No difference was observed between the green frog and bull frog. The muscle was pressed between silver electrodes. It was found that a film of AgCl did not lower the impedance. The measurements were made with a Wheatstone bridge with equal ratio arms. By balancing the tissue against a circuit that would remain in balance with change of frequency, the type of circuit in the muscle was imitated and was: a capacity (C) in parallel with a resistance r and the whole in series with another resistance R. Green frog thigh muscles (unstimulated) balanced (with equal ratio arms) against a circuit with $r = 226$ ohms, $C = 0.145$ microfarads and $R = 125$ ohms at 1 K. C., 2 K. C. and 3 K. C., whereas if C was increased or decreased and r and R changed to balance at one frequency it did not balance at another. For instance if C is set at 0.070 mf. at 1 K. C., $r = 300$ ohms and $R = 15$ whereas at 3 K. C. $r = 290$ and $R = 20$. If C is set at 0.245, at 1 K. C. $r = 200$ and $R = 200$, but at 3 K. C. $r = 225$ and $R = 210$.

TABLE II.

Animal	Kilocycles	Physiological State	Microfarads C	Ohms R
Dogfish	2.5	u	—	500
		s	—	489
Bullfrog	0.9	u	3.24	285
		s	3.60	267
,,	1	u	2.45	309
		s	2.50	299
,,	1	u	2.50	300
		s	3.00	290
,,	2	u	0.935	286
		s	0.935	262
,,	2	u	0.930	318
		s	0.960	272
,,	3	u	0.500	270
		s	0.550	250
Green frog	2	u	1.00	317
		s	1.10	300
,, ,,	2	u	1.10	300
		s	1.14	297

u = unstimulated. s = stimulated.

In another experiment with bull frog muscle at all 3 frequencies r = 198, C = .195 and R = 98.

If *r* is made zero a balance may still be obtained but varies with frequency as shown in the following table (together with the change in resistance and capacity with stimulation).

It may be seen from the table that the resistance invariably decreased on stimulation. Change of shape may have an effect on resistance. Since the muscle placed between the electrodes had muscle fibers extending in various directions, it was thought that the effects of change of shape of the different fibers might tend to neutralize one another. The fact that there was always a decrease in resistance is considered as indicating that there was a decrease in resistance of the individual muscle fibers. The changes in capacity are not so regular.

3773

The Effect of Hypercalcemia on the Creatin Output in Myasthenia Gravis.

HILDING BERGLUND, GRACE MEDES AND ANNE LOHMANN.

From the Medical Service of the University Hospital, Minneapolis.

The discovery by Fiske and Subbarow[1] of muscle phosphocreatine as a labile compound with a definite relation to the functional condition of the muscle, again awakened the interest in those diseases of the (neuro)muscular system, which are characterized by creatinuria, *viz.*, progressive muscular dystrophy and myasthenia gravis. The occurrence of acute attacks of muscular incompetence in myasthenia gravis versus the more steadily yet slowly progressive course of the muscular dystrophy made us choose the former disease for our experiments. The question whether the escape of the creatine from the muscle is due to an error of metabolism of the sarcoplasm or to an abnormal permeability of the sarcolemma presents itself. Through the parathyroid hormone preparation by Collip it has become possible to influence the blood calcium level sufficiently for the carrying out of cell-membrane permeability experiments in human subjects.

We report briefly an experiment designed to demonstrate the effect of hypercalcemia on the creatin output in myasthenia gravis. The patient was 45 years, with myasthenia gravis of one year's duration, the diagnosis having been made in the neurological divi-

sion. This patient is also the chief subject of the observations reported in the following paper. During the experiment the patient was on a creatine- and creatinine-free diet. The usual Folin methods were used for the urine determinations, and Clark's and Collips' method[2] for the plasma calcium.

TABLE I.

Day of experiment	Procedure	Serum Ca per 100 cc.	Creatinine per 100 cc. urine (daily average)	Creatine per 100 cc. urine (daily average)
		mg.	mg.	mg.
1-12	Fore period	10.6	1.05	.65
13-16	5 cc. 10% $CaCl_2$ intravenously daily	11.0	1.12	.55
	13-15 day	10.6		
17-19	Intermediary period		1.11	.65
20-29	Units of parathyroid hormone each day: 18, 20, 55, 55, 60, 75, 50, 75, 75. Ca-lactate, 9 gm. daily	10.7		
		12.9	1.16	.57
		13.3		
30-36	Afterperiod		1.06	.58

The table is self explanatory. The blood calcium shows no increase on intravenous administration of $CaCl_2$ alone. On subcutaneous administration of parathyroid hormone (Lilly) in combination with large doses of Ca lactate by mouth (9 gm. a day) the blood calcium slowly rose from 10-11 mg. to 13.3 mg. per 100 cc. serum. At this point the patient complained of headaches and the hypercalcemia was no longer kept up. During no period, neither during the intravenous Ca treatment nor during the parathyroid treatment did the creatine or creatinine figures show any significant changes.

From the point of view put forward in the introduction we consider the experiment worth reporting.

[1] Fiske, C. H., and Subbarow, Y., *Science*, 1927, N. S. lxv, 401.

[2] Clark, E. P., and Collip, J. B., *J. Biol. Chem.*, 1925, lxiii, 461.

3774

Analysis of Morphological Blood Changes in Pernicious Anemia Following Administration of Liver.

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The introduction by Minot and Murphy[1, 2, 3] of the liver diet in the treatment of pernicious anemia has supplied experimental medicine with a hitherto unequalled method of studying blood formation. Three general types of anemia have been produced experimentally, secondary anemia due to hemorrhage, the aplastic anemia due to destruction of the blood forming element of the bone marrow, and the hyperchromatic anemia produced by certain poisons. The fact which differentiates the condition in pernicious anemia from the types of anemia which have been experimentally produced, is that in pernicious anemia a blood is available which shows a low spontaneous regenerative activity and yet the bone marrow is not aplas-

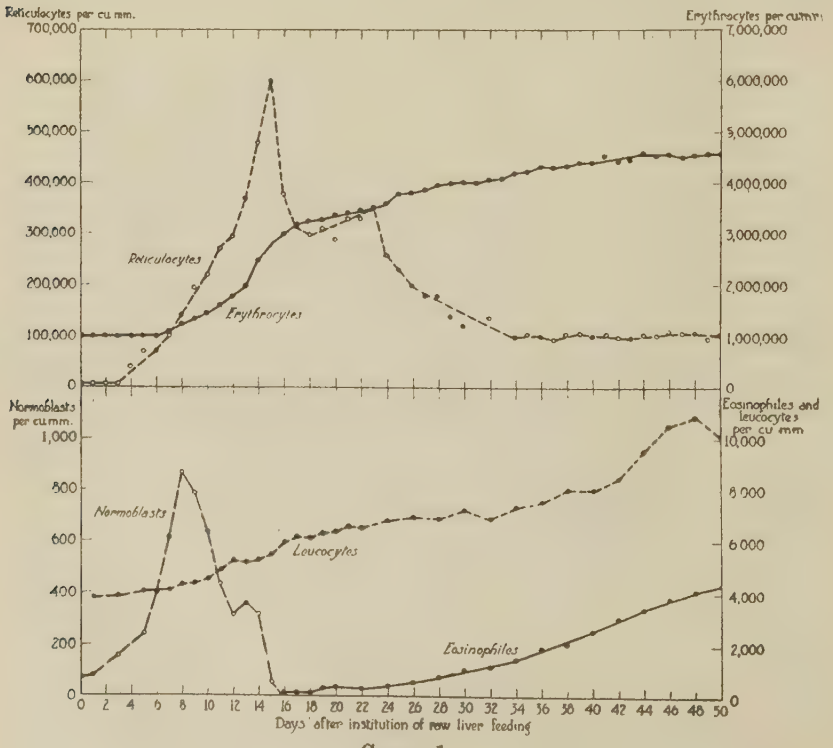

CHART 1.

tic. Minot and his co-workers have shown that after about two months of the liver treatment, the morphological blood picture of pernicious anemia has returned to practically normal.

A detailed study of the morphological blood features in a case of pernicious anemia is presented in figures 1 and 2. The patient is a married woman 52 years of age presenting the classical picture of the disease. Before the liver diet (all liver given raw) the morphological picture was observed for 5 days, ascertaining that no signs of blood-forming activity were present. There is in our graphs no curve for megaloblasts. Whether they occur or not is a question of more than momentary interest. The megaloblast is a cell which is normally produced in the blood islands around the yolk sac and in the connecting stalk of the embryo, and pathologically occurs only occasionally in pernicious anemia and severe toxic anemias. It was first fully described by Ehrlich,[4, 5] who also sharply differentiated between the megaloblast and the normoblast. Later investigators seem to have overlooked the finer morphological characters which distinguish the two types of cells and base their differentiation chiefly upon size, which is no criterion, for normoblasts frequently

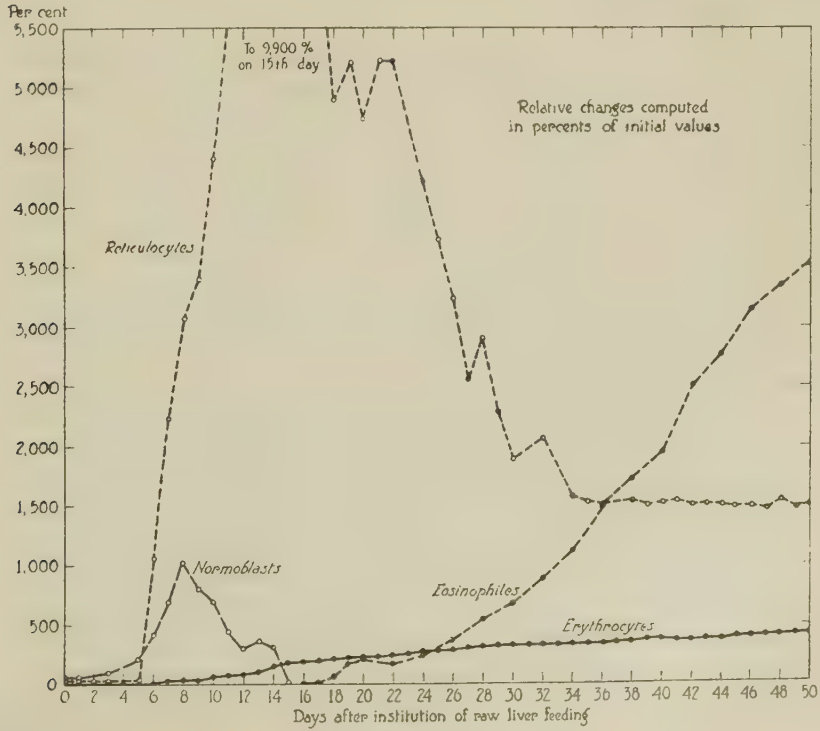

CHART 2.

are as large as, or larger than megaloblasts. Naegeli[8] claims that megaloblasts can be found in practically all cases of pernicious anemia and other investigators believe that the megaloblasts must be present in order to establish the diagnosis of pernicious anemia. Out of a series of 14 cases presenting a typical clinical, as well as morphological picture of pernicious anemia, megaloblasts were found in only two cases and in these instances none were found after the liver treatment had been instigated. It would seem surprising if a regenerative process leading to a normal blood picture were ushered in by an exaggeration of the most unusual pathological feature of the erythrocyte.

At the time the liver diet was started the total number of normoblasts per cu. mm. of blood was 78. Immediately after the liver was started they increased rapidly so that by the 8th day the total number was 870 per cu. mm., after which there was a gradual decline, until by the 16th day they had practically disappeared. Figure 2 represents a graph of the percentage increase, using the value before liver was started as unity and from this calculating the increase in percentage over unity. The highest percentage for the normoblasts is 1015 on the 8th day.

The reticulocyte curve began to rise 4 days after treatment was started and reached its peak on the 15th day, at which time the normoblasts had practically disappeared. The increase was 9,900% on the 15th day. This represents the highest percentage increase of any of the formed elements, giving a mathematical expression of the happy stroke of Minot and Murphy in choosing the reticulocyte count as the chief check on the effect of the liver feeding. There was a slight secondary rise in the normoblast curve on the 13th day and a similar one occurred for the reticulocytes on the 23rd day. At first this was thought to be an individual variation. However, a similar rise has been found in other cases, but as yet its significance is not established.

The total red count remained relatively constant until the 6th day after the diet was started and then there was a marked increase up to the 18th day. From this time on the count rose more slowly up to normal. The increase in erythrocytes was about 410%, which was reached 50 days after liver was started. Comparing these curves with the graphs of the reticulocytes, it will be found that at the time of the most rapid increase in reticulocytes there is also the most rapid increase in total red cells. It is also shown that, although the reticulocytes offer a good means of estimating the regenerative activity of the bone marrow, they do not represent the only method of erythrocyte production. Thus the total reticulocytes increase to slightly

over 600,000 per cu. mm., while at the same time the total red cells increase from below 1,000,000 up to more than 3,000,000 cells per cu. mm. Therefore, many mature erythrocytes are being released from the bone marrow along with the less mature reticulocytes. The rate of increase of erythrocytes is correlated with the reticulocyte production, for after the peak of reticulocytes is reached there is a slowing down of the rate of red cell production.

From the time the liver treatment started, up to the 18th day, the percentage of eosinophiles remained relatively constant. From this time on they increased from approximately 2% to about 48% of the total number of leucocytes. Since the eosinophilia begins just after the peak of reticulocytes has been reached and at the time the rapid increase in the red count has diminished, this may possibly be regarded as a reaction of the body to an overdose of liver.

Although the morphological blood picture returned to normal following the liver treatment it was found that the so-called "pernicious anemia neutrophiles" remained. This has likewise been observed in other cases. Whether this deviation from normal will ultimately disappear has not been established, but it is of aid in the diagnosis of pernicious anemia after the blood picture has become essentially normal.

Conclusions—1. Megaloblasts and normoblasts are morphologically different types of cells. 2. Megaloblasts are not essential in the establishing of the diagnosis of pernicious anemia. 3. The liver diet causes a response in the circulating blood in the form of an appearance of (a) normoblasts, (b) reticulocytes, (c) mature erythrocytes, (d) eosinophiles. 4. The progressively increasing eosinophiles may be an expression of an overdosage of liver.

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[1] Minot, G. R., and Murphy, W. P., *J. Am. Med. Assn.*, 1926, lxxxvii, 470.

[2] Minot, G. R., and Murphy, W. P., *J. Am. Med. Assn.*, 1927, lxxxix, 759.

[3] Minot, G. R., and co-workers, *Transact. Ass. Amer. Phys.*, 1927, xlii, 83.

[4] Ehrlich, P., De- und Regeneration roter Blutscheiben. Verhandl. d. Gesellsch. d. Charite'ärzte. 1880, June 10 and December 9. Quot. fr. Lazarus.

[5] Lazarus, A., *Nothnagel's Handbuch d. Spez. Path. u. Ther.* Vol. viii. Die Anaemie p. 1 und 107. Wien, 1913.

[6] Naegeli, O., Blutkrankheiten u. Blutdiagnostik. 4th edit., 1923.

3775

An Unknown Reducing Urinary Substance in Myasthenia Gravis.

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In the course of the determination of urinary inorganic phosphate by the method of Fiske and Subbarow[1] in a case of myasthenia gravis it was observed that the phosphomolybdic acid became reduced and an intense blue color developed before the reducing agent, aminonaphtholsulfonic acid, was added. The same immediate reduction of the phosphomolybdic acid did not take place after the acid digestion in the course of the total phosphorus determination. It was also observed that occasionally in this particular urine the inorganic phosphate figures slightly exceeded the values for total phosphorus. Attempts to elucidate this phenomenon have led to our present conception that we deal with a hitherto unknown reducing urinary substance.

We have obtained the same color reaction from the urines of two other cases of myasthenia gravis, but have failed to demonstrate the same in one case of muscular dystrophy and in one case of muscular atrophy, diseases in which as in myasthenia gravis a creatinuria is or may be present. Of our second and third cases of myasthenia gravis, which at the present show a milder clinical picture than our first case and from which only random samples of urine were obtained, none developed as deep a color as our first case. In one we designate the color as weak but definite, in the other as of moderate intensity. In normal urines we have never observed this reaction, which also must be in accordance with the experiences of Fiske and Subbarow. The only other condition in which we have observed a similar behavior is alkaptonuria.

The new compound reduces alkaline copper solution more slowly than does glucose, thus in the Folin and Berglund method for the sugar of normal urine considerable color development takes place on boiling beyond 8 minutes. With this method and 8 minutes boiling the 24 hours urine of our patient shows a reducing value corresponding to 2.5-3 gm. of glucose. On starvation as well as on high carbohydrate feeding these values vary only as much as may be explained from the corresponding known variations in the so called sugar values of normal urines. High meat diet seems to cause a slight increase.

The compound present does not ferment, does not increase its re-

ducing value of acid hydrolysis as used in the Folin and Berglund method, does not give the phloroglucin or orcin tests for pentoses. An osazone could not be obtained. The compound does not reduce alkaline picrate at room temperature nor interfere with the matching of the color in the creatinine determination. During picric acid hydrolysis as used in Folin's creatine determination an alteration is produced which somewhat throws off the color developed in the subsequent creatinine determination.

Attempts to isolate the compound have revealed the following facts: it is not precipitated by phosphotungstic acid, by tannic acid, by mercuric chloride nor by neutral lead acetate. With basic lead acetate it is partly carried down with the lead precipitate, from which it may again be removed by washing with water. It is nonvolatile and cannot be distilled from water by steam distillation. It is somewhat soluble in ethyl alcohol, ether, chloroform, acetone, toluol, xylol and petroleum ether. It may be extracted by alcohol from the urine after saturation with ammonium sulphate. It becomes oxidized in the course of weeks on exposure to air. It is oxidized by hydrogen peroxide, bromine, ferric chloride or by heating with nitric or sulfuric acid. It is destroyed by mercuric nitrate. The compound reduces phosphomolybdic acid in both acid and alkaline solution, apparently more rapidly in acid solution. It reduces phosphotungstic acid in alkaline solution.

For further differentiation of this compound against homogentisic acid it may be stated that this urine does not darken on the addition of ammonia and that our compound does not reduce ammoniacal silver solution at room temperature. Homogentisic acid reduces phosphomolybdic acid more rapidly, almost instantly, while the complete reaction with our compound requires several minutes. Both give a weak Millon's reaction. So far no precipitates have been obtained with saturated sodium bisulphite nor with semicarbazide. Schiff's aldehyde reaction was positive to a considerably greater extent than with any normal urines tested. The color obtained on our urine corresponded approximately to the color given by formaldehyde at a dilution of 1:50,000. It might here be recalled that formaldehyde does not reduce phosphomolybdic acid.

[1] Fiske, C. H., and Subbarow, Y., *J. Biol. Chem.*, 1925, lxvi, 375.

New York Meeting.

College of Physicians and Surgeons, December 21, 1927.

3776

Effect of Glucose on the Ketone Body Excretion in Fasting Depancreatised Dogs.

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It is a familiar fact that the administration of carbohydrate to fasting or diabetic patients, or to phlorhizinized dogs,[1] immediately abolishes the ketosis, but the possibility of a similar effect of carbohydrates on the ketosis of completely depancreatised, fasting dogs has not hitherto been investigated. Such observations are recorded here.

Method. Dogs were completely depancreatised and were then fed twice daily, with meat, raw pancreas and sugar, and injected with insulin, until the wound had healed. To induce the diabetic state food and insulin were withheld. Urine was collected from the cage and by catheter at intervals of 12 hours. Acetone bodies in the urine were determined by the Van Slyke method, urinary glucose by the Shaffer-Hartmann, and nitrogen by the Kjeldahl method. The results of two experiments are shown below:

TABLE I.

Dog 1. Wt. 6 kg.

Period of fasting	Nitrogen	Glucose	D/N	Total ketones	Remarks
	gm.	gm.		gm.	
1st 12 hrs. 3rd day	3.45	24.0	6.9	0.16	
2nd 12 hrs. 3rd day	4.56	16.3	3.6	0.56	
					50 gm. sucrose by stomach tube
1st 12 hrs. 4th day	5.42	46.5	8.6	1.36	

TABLE II.

Dog 2. Wt. 6 kg.

Period of fasting	Nitrogen	Glucose	D/N	Total ketones	Remarks
	gm.	gm.		gm.	
1st 12 hrs. 3rd day	2.38	10.9	4.6	0.49	
2nd 12 hrs. 3rd day	2.75	13.4	4.9	1.24	
					45 gm. sucrose by stomach tube
1st 12 hrs. 4th day	3.50	54.7		1.76	
2nd 12 hrs. 4th day	3.37	12.7	3.72	1.71	

It had previously been found by one of us[2] that the excretion of the acetone bodies on the third and fourth days of fasting in 6 depancreatised dogs was as follows:

Period	Ketone body excretion					
	1	2	3	4	5	6
2nd 12 hrs. 3rd day	0.25	0.72	0.12	0.08	0.78	0.33
1st 12 hrs. 4th day	0.42	1.12	0.53	0.58	0.46	0.39

The results show that the antiketogenic action of carbohydrate does not obtain in the animal organism in the complete absence of insulin, and in view of the known fact that glucose stimulates the secretion of insulin in the normal animal, it is interesting to consider the possibility that the antiketogenic action of administered carbohydrate in *Diabetes mellitus* and in phlorhizin poisoning may be due to the ensuing secretion of insulin, which, it has been shown, lowers the ketone bodies of the blood following its injection to fasted, depancreatised dogs.[3]

[1] Wierzuchowski, M., PROC. SOC. EXP. BIOL. AND MED., 1925, xxii, 425.

[2] Chaikoff, I. L., *J. Biol. Chem.*, 1927, lxxiv, 203.

[3] Chaikoff, I. L., Macleod, J. J. R., Markowitz, J., and Simpson, W. W., *Am. J. Physiol.*, 1925, lxxiv, 36.

3777

Return of Vision and Other Observations in Replanted Amphibian Eyes.

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Since Koppanyi[1] reported that vision returned in certain vertebrate eyes after replantation and transplantation, conflicting results have been published which show that return of vision as well as

other related problems is still unsettled. Technique in operation and handling of the replanted or transplanted eyes has much to do with its subsequent conditions. In such problems it is of value to know the existing conditions in both young and adult animals. We are pursuing the problem with this in mind.

Operations on *Amblystoma punctatum* larvae (19 to 23 mm.) and adult *Diemyctylus viridescens* are here reported. Chloretone anesthetic was used. The eyes were excised by finely sharpened iredectomy scissors and forceps manipulated under the compound dissecting microscope. Very little hemorrhage was encountered.

Return of circulation was first noted within superficial vessels in the iris, in *Amblystoma* larvae as early as 24 hours after operation, in *Diemyctylus* as early as the 5th day. Resorption of the eye could usually be predicted by known injuries to the bulb at the time of operation, or by too long use of anesthetic or a too strong anesthetic. Slight reduction in the size of the eye after operation always persisted in *Diemyctylus,* the maximum reduction being attained in about a month. In *Amblystoma* larvae reduction did not always take place. When it did there was usually rapid recovery. The cornea occasionally became opaque, but in most cases became clear about the time of observed return of circulation. All specimens of both types of amphibians kept several months after operation showed corneal reflex, when only the cornea was touched by a fine hair. In many *Diemyctlyli,* 7 weeks after operation replanted eyes readily retracted when the cornea was so stimulated.

Return of ocular movements were found in both types of replanted eyes, in *Amblystoma* as early as 9 days after operation. Some replants showed restricted movements but in most cases movements seemed normal. The pupil in replanted larval eyes slightly dilated shortly after operation, later approaching normal size. In *Diemyctylus* the pupil gradually became maximally contracted within a month after operation. Later it approached normal size. The iris often cupped inward and appeared darkly pigmented. This was not observed in larval eyes. Replanted eyes showed pupillary reactions to light both in the presence and absence of return of vision and optic nerves. One *Amblystoma* and one *Diemyctylus,* in each of which there was return of vision and an optic nerve, showed no pupillary reactions.

Extreme care was taken in tests for return of vision to eliminate olfactory, gustatory, tactile and mechanical disturbances such as jarring or touching the aquarium. To test visual response, a piece of red rubber impaled on wire was moved about outside of a tightly

covered aquarium. Tests were made many times for several weeks by the investigators and disinterested observers. Each *Amblystoma* was metamorphosed when final tests for return of vision were made. Vision in the normal eye, if present in the tested operated animal, was eliminated either by a light-proof patch of celloidin and lamp-black placed over it, or it was extirpated. For comparison, controls were used, *viz.*, animals (a) with 2 normal eyes, (b) with 2 eyes extirpated and (c) with 1 normal eye and 1 extirpated.

Most animals with only the replanted eye exposed reacted like animals with normal eyes exposed. We have also found return of vision in replanted adult *Amblystoma* eyes. Although our data at present are incomplete, we have found that it can take place as early as 3 months after operation. They snapped vigorously at the moving source of stimulus or followed it about. Microscopic examination of such specimens showed an optic nerve from the bulb to and through the chiasma. Tests for return of vision proved negative (like blind animals) in a few cases and microscopic examination in these revealed no optic nerves. In some cases the regenerated optic nerve seemed normal *(Amblystoma),* in most cases *(Amblystoma* and *Diemyctylus)* smaller aud usually tortuous.

The retinae of replanted eyes in both types of amphibians showed reduction of ganglion cells to about one-half normal number. Small spots often lacked these cells. *Amblystoma* killed within a month after operation began to show this change.

Phototactic responses, similar only to normal were obtained in only those cases *(Amblystoma* and *Diemyctylus)* where there was return of vision.

One case of a replanted larval eye showed return of vision in which the eye was rotated 90° anteroventrally, and 2 cases, in which the eye was rotated 180° anteroposteriorly, showed neither return of vision nor regenerated optic nerves. The eyelids developed in the rotated position. The retinae showed conditions similar to replanted larval eyes normally rotated.

[1] Koppanyi, T., *Arch. f. mikr. Anat.*, 1923, xcix, 15-63.

3778

Morphological Elements Present in the Tubercle Bacillus Cultures.

L. DIENES.

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When tubercle bacillus cultures are ground with water and centrifuged, the largest part of the bacteria is easily sedimented, but a turbid suspension remains which cannot be cleared, even with prolonged centrifugalization. With the Sharpless super centrifuge a nearly clear fluid is obtained, and the filtration through infusoria earth or paper pulp gives a perfectly clear filtrate. After filtering the thoroughly centrifuged suspension a viscous material remains on the filter, which material can be easily distributed in water. Examination of this revealed the following characteristics: Stained according to the method of Ziehl Neelsen it takes the counter stain, very few acid-fast bacteria being present in it. More thorough examination of the preparations (the best pictures are obtained in slides not decolorized after the carbolfuchsin staining) shows that the non-acid fast material consists of bacteria and small granules. These forms, both the bacteria and the granules, even when the preparations are not decolorized, are stained neither with fuchsin nor with methylene blue, but can be seen as a negative picture between small amounts of slightly red or blue material. In the dark field numerous small granules are visible between larger forms. We obtained the best information concerning the form and size of the element in smears with argyrol. These non-colorable forms were found in large amounts in the broth cultures of 5 strains and in the culture of a freshly cultivated strain on egg medium. The described forms are present in the cultures, and are not produced through the grinding of the bacteria. This is shown when a small piece of culture is spread with gentle rubbing on a slide. The coloration of Ziehl-Neelsen and counter-staining with methylene blue reveals the presence of large numbers of them in the preparation. It is necessary to examine several areas of the slide, because in some places only acid-fast bacteria are visible, while in other places only few acid-fast forms are found in a slightly blue material. In one case, grinding about 3 gm. (dry weight) bacteria several times with water, we obtained about 0.3 gm. of this material.

The filter residue, the morphological characteristics of which have been described, has high dry material content. Its chemical examination revealed that it contains more nitrogen than the washed bac-

teria (8% and 5.6%). It contains more methyl alcohol and ether soluble material (32% and 28.5%) than the washed bacteria. After boiling 6 hours with 5% HCl we could not find reducing sugar. The washed bacteria gave about 10%. Upon the addition of acids it gives a coherent precipitate. In one case at pH 5.2 a slight, and at pH 5.0 complete precipitation occurred.

The filter residue is a very strong antigen, both *in vivo* and *in vitro*. The antigen unit in the complement fixation test was 1/30 of the antigen unit of washed bacteria. The washed bacteria cannot be well suspended in saline. The sera of guinea pigs in the course of the tuberculous infection show complement fixation more often and of higher titer with it than with any other antigen we examined. This is also the case with most of the immune sera. The material contains less of the carbohydrate precipitable substance than the washed bacteria (extraction at 100 C° with 2% Na_2CO_3 and 2% $NaC_2H_5O_2$) and somewhat more methyl alcohol soluble antigen. It gives no or only very little agglutination. The sera obtained with this material differ qualitatively from the sera obtained with the washed bacteria.

It is known that in old cultures large numbers of bacteria are dead. As tubercle bacillus cultures are always old, it is possible that the forms described in this paper are the dead bacteria. If this is the case it is remarkable that besides the loss of colorability and fragmentation, the precipitability with acids and the antigenic properties have undergone a change which does not occur in artificially killed bacteria. Certain antigenically active constituents of the cells are newly formed or become preponderant.

Do the bacteria undergo a similar change in the organism? Are the granules in the cultures alive? Do they represent the so-called filtrable forms of the tuberculous virus which plays at present a great rôle in French literature? But even without thinking of the eventual connection with important biological problems, the presence of morphological elements with changed chemical and antigenic properties is of importance for the chemical and antigenic investigation of the bacteria. The admixture of these forms, even in small proportion, to the bacillary extracts, can considerably change their antigenic properties.

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Biometric Studies Upon Development and Growth in Amblystoma Punctatum and Tigrinum.

E. M. PATCH. (Introduced by J. S. Nicholas.)

From the Osborn Zoological Laboratory, Yale University.

Measurements of *Amblystoma punctatum* and *tigrinum* have been made in order to secure data upon the normal rate of growth and upon the modifications of this growth rate by experimental procedure. Data show that the rate of enlargement during embryonic stages is dependent upon the temperature, the size at any named stage being correlated with the size of the egg. Length increase in the embryo is made at the expense of the other dimensions. The curve of absolute increments of length when plotted against time during embryonic development is S-shaped, terminating at the end of this period.

The mean lengths of *Amblystoma punctatum, tigrinum,* and *Axolotls* at this point of development are 15.61 mm., 14.07 mm., and 11.96 mm., respectively. Tables for mean length, standard deviation coefficient of variation, and the probable errors of these quantities demonstrate the value of the criterion used.

Growth following the embryonic period is dependent upon food—quality, quantity, and frequency of feeding. Where feeding is alike, size relations of the embryonic period hold for larval growth. No food has been found as adaptable for early use as the natural diet which consists of small aquatic forms. Beef-liver can be used at early stages and produces a remarkable acceleration of growth. Of the tissues tried, kidney is second in value.

In the later larval development and adult life, beef muscle produces greater growth than liver; at these stages, liver feeding is attended by excessive glandular production, by lack of growth, and often with loss of appetite. Death sometimes follows. There is a weakening of normal peristaltic action, which can be compensated for, partly at least, by use of agar. Beef muscle is of poor value for early larval growth and development. Here, as later, there is a lack of pigmentation with this food. In later larval and adult life, while the growth made on muscle is large, the mineral deficiency is evident—especially the calcium lack as shown by the tetanic condition.

Feeding on earthworms, with cuticle broken and digestive contents removed, produced little growth; the animals so fed are, however, apparently normal. *Enchytraei* (white worms) as food give a good growth rate; but the animals so fed, though large and well-formed,

do not metamorphose. Animals fed on beef muscle will not metamorphose without addition of vitamins or minerals to their diet. Metamorphosis occurs successfully with liver and with earthworms, as is true with a mixed diet of the meats and worms. The latter, started in later larval life, produces the best growth of all diets. All of the reactions to foods are modified by the quality, quantity, frequency, and duration of the initial diet.

Use of synthetic diets emphasized the need for minerals and vitamins, the greater growth with vitamin A, showed greater growth with deptrin present, the value of low fat content, the preference for beef muscle powder as protein basis rather than the powdered liver, egg-white, egg-yolk, casein, or klim—though growth was made with all of these. The diets were more useful with older, larger larvae. Younger larvae showed different relative reactions.

During larval life the size relations of *Amblystoma punctatum* and *tigrinum* were reversed, so that at the time for metamorphosis the ratio was approximately 1:2 in the order named. Larval life gave another S-cycle to the growth curve, this cycle terminating at the time of metamorphosis. A third life cycle, beginning at this point, is probably related to the development of sexual maturity. Plotting of the cube of length against weight, for measurements made on adults, shows the first quantity to be a linear function of the second.

Hibernation of adults was followed, on return to warm temperature and food, by a remarkable acceleration of growth until the size characteristic for the stage of development was reached or surpassed. The tail became proportionally shorter during the hibernation period, with return to normal proportion after renewal of growth. The index of build; weight/length, (Bardeen *et al*), was slowly decreased with increase of size.

3780

The Effect of Glucose on Ketosis.

W. A. SELLE. (Introduced by N. R. Blatherwick.)

From the Potter Metabolic Clinic, Santa Barbara Cottage Hospital, Santa Barbara, and the Department of Physiology, Stanford University.

During a study of the influence of insulin on fat and ketone bodies in the blood of depancreatized dogs, several observations were made on the effect of glucose, without insulin, on ketosis. Since clinical

experience has shown that glucose given to patients in diabetic coma often ameliorates the symptoms of ketonuria, it is of interest to determine whether or not the same effect can be demonstrated on animals made diabetic by removal of the pancreas.

Observations on animals under the influence of phlorhizin show that the administration of glucose is followed by a decrease or disappearance of ketone bodies, a phenomenon similar to that noted by the clinician.

Whatever may be the effect of glucose on animals rendered diabetic by the drug phlorhizin, a similar effect is not necessarily to be expected on depancreatized animals, for the physiological conditions of the two experimental animals are quite unlike. Although the exact effect of phlorhizin on the animal has not been definitely established, recent investigations indicate that there is no impairment in the ability of the tissues to metabolize glucose when present in normal quantities, and that its action is largely renal. In the depancreatized animal the tissues loose their power of metabolism because of the lack of insulin. Therefore studies of the ketolytic influence of glucose on phlorhizinized animals throw little or no light on any effect, or lack of effect, that it may have on depancreatized animals.

The present observations were made on 4 totally depancreatized dogs kept alive by the administration of insulin twice daily until recovery from the operation and resulting complications. Results of the operation were confirmed at autopsy. The degree of ketosis developed in the animals after the withdrawal of insulin varied, depending largely on the nutritive condition of the animal and the time lapsing between the withdrawal of insulin and the beginning of the experiment. As the animals were starved for 3 or 4 days prior to the administration of glucose, the ketone bodies formed are assumed to be derived entirely from the breakdown of the animals' tissues. Only 2 animals were allowed to develop a severe ketosis (3 mg. or more of total ketone bodies per cc. blood), the other 2 had a pronounced but not advanced ketosis (both registered between 1.5 mg. and 2 mg. of total ketone bodies per cc. blood). In 3 of the 4 experiments, acetone plus aceto-acetic acid was higher at the beginning of the experiments, and remained higher throughout, than hydroxy-butyric acid. In the fourth, hydroxy-butyric was slightly higher than the combined acetone and aceto-acetic acid.

After the initial blood samples had been taken, glucose was given, either by mouth (2 cases) or hypodermically (2 cases), and blood was drawn thereafter at 2 or 3 hour intervals for a period of 8 or

10 hours. The amount of glucose given by mouth was not the same for the two animals; one received 75 gm. and the other 60 gm. The animals receiving glucose hypodermically each received 16 gm. of a 20% solution injected subcutaneously.

In no case did any of the ketone bodies diminish after glucose was given. In two cases an increase was observed in the acetoacetic acid during the experiment; in one case, hydroxybutyric acid also increased.

Since these observations indicate that glucose has no effect on ketonemia of animals deprived of their pancreas, it is interesting to account for its effect on the diabetic patient. Dr. J. J. R. Macleod suggests that it may be due to stimulation of islets of the pancreas not yet involved in the disease process; the resulting increased production of insulin is assumed to more completely metabolize the ketones. Since no islets are left in the depancreatized animal, this theory seems in harmony with the results here obtained.

3781

Expulsion of Injected Solute by Contractile Vacuole of Amoeba.

RUTH B. HOWLAND AND HERBERT POLLACK.
(Introduced by Robert Chambers.)

From Washington Square College and Cornell University Medical School.

Although it has long been the presumption that the contractile vacuole expels substances in solution in the endoplasm of the protozoon, the actual taking up and expulsion of a definite solute by the organoid has never been demonstrated. This now has been accomplished by means of the micrurgical apparatus.

If a moderate amount of saturated aqueous solution of picric acid is injected into an ameba *(Amoeba dubia)* the course taken by the solute may be traced by its yellow color. The effect on the cytoplasm has already been described by Pollack.[1] Though a part of the colored region is often injured by the pipette and thereupon pinched off by the ameba, a certain quantity of the solute diffuses into the remaining endoplasm before this occurs. This is taken up by the vacuole, the intensity of the yellow color of the vacuolar fluid increasing in proportion to the fading out of color in the endoplasm.

When a 2% solution of picric acid in 95% alcohol is injected, diffusion throughout the endoplasm is much more rapid, and the yellow color appears more quickly in the vacuolar fluid. In the

small percentage of cases where the injected region is not pinched off and the entire amount of the solute is retained, the fluid in the enlarging vacuole becomes an intense yellow. Such vacuoles progressively become very flaccid. Contact with the least obstacle, or stress exerted by endoplasmic currents easily causes their deformation. Their limp membranes may temporarily infold deeply, and the vacuoles often appear bean-shaped, long ovoid, or pyriform. Systole of these flaccid vacuoles is delayed, and a new vacuolation center appears and functions. In the meantime the original vacuole is carried about, gradually becoming more turgid and uniformly spherical, and finally contracts.

When smaller amounts of picric acid are injected, or when the ameba rids itself of the larger proportion of the solute by pinching off the injected region, the vacuole does not show any evidence of flaccidity but remains spherical and turgid although its contents are appreciably yellow. It increases in size at a rate similar to that subsequent to injections of distilled water,[2] and finally ejects the yellow fluid. After each systole, the collected fluid becomes successively paler until both endoplasm and vacuole have entirely lost the yellow color.

[1] Pollack, H., PROC. SOC. EXP. BIOL. AND MED., 1927, xxv, 145.

[2] Howland, R. B., and Pollack, H., *J. Exp. Zool.*, 1927, xlviii, 441-458.

3782

Hyperergic Tissue Response to Non-Hemolytic Streptococci.

CLIFFORD L. DERICK AND HOMER F. SWIFT.

From the Hospital of the Rockefeller Institute for Medical Research.

Two years ago in collaboration with Andrewes[1, 2] we described a peculiar response in the skin of rabbits to the inoculation of certain non-hemolytic streptococci. This was termed the secondary reaction because it occurred after the primary reaction to the inoculation had subsided. It was shown[3] not to correspond in its various phases with the Arthus phenomenon which is induced in rabbits by immunization and subsequent skin injection with various coagulable proteins. We, therefore, suggested that if this were an allergic phenomenon it was of the type seen in tuberculosis. Our subsequent investigations have been guided by this hypothesis.

The object of this report is to describe some of the concomitant phenomena of the secondary reaction and suggest their significance.

Eye Reaction: If the locally anesthetized cornea of a rabbit, which has shown a well marked secondary reaction, is lightly scarified and a drop of broth culture sediment of homologous streptococci is instilled into the conjunctival sac, there usually follows a distinct interstitial keratitis, characterized by increasing congestion of the ocular conjunctiva, turbidity of the cornea, and ingrowth of vessels from the sclerocorneal junction; this is followed by a gradual subsidence of signs until only a slight scar remains. The signs usually begin 24 to 48 hours after inoculation and last from 4 to 8 days, sometimes longer. Occasionally the reaction is delayed several days. Normal rabbits and rabbits inoculated intravenously have never shown keratitis following similar eye inoculations.

Skin Reaction: Inoculation of the skin of a rabbit, showing a secondary reaction, with a small dose of culture, *i. e.,* 0.0001 cc. is followed by a local lesion much more intense and of distinctly longer duration than is seen in a normal rabbit. This lesion often has a dull red color comparable with that seen in a nodular syphilide or in lupus nodules. Reinoculation of the skin of a secondary reacting rabbit with larger doses is followed by marked edema and other signs of a more intense reaction than are shown by normal rabbits inoculated with similar amounts. These increasingly severe skin reactions are very comparable with local tuberculin reactions in man, or with the response to cutaneous inoculation with tubercle bacilli in tuberculous animals.

Lethal Reactions: If rabbits shortly after the development of secondary reactions are injected intravenously with doses of homologous streptococcus viridans, which are well tolerated by normal rabbits, they will often die within 24 to 48 hours. They show on autopsy enlarged hemorrhagic lymph nodes, multiple hemorrhages into the bone marrow, a much enlarged thymus filled with petechial hemorrhages and at times hemorrhages into the endocardium and myocardium. Sometimes animals show extreme prostration but recover; if these animals are autopsied shortly after recovery they show similar but less marked lesions. This lethal reaction is similar to that seen in tuberculous animals following intravenous injection of large doses of tubercle bacilli or of tuberculin.

From the foregoing observations it is evident that the secondary reaction is accompanied by a generalized hyper-reactive state of the animal, hence the secondary reaction itself is probably a concomitant phenomenon of this state, and due to the reactivity of the tissues locally to some reaction stimulating substance that has remained at the original site of inoculation. It is true generalized *hyperergy* and for convenience the condition of these animals is designated as the

hyperergic state. This state is roughly proportional to the size and number of inoculations. It can be increased up to a certain limit if the animal is repeatedly inoculated at intervals of 5 to 10 days. Its induction appears to be due to the presence of one or more lesions some place in the body. It is not specific in the sense of serum immunological specificity, for an animal made hyperergic with one strain of non-hemolytic streptococci is also hyperergic to other strains which show cultural and agglutinative differences. It seems probable, therefore, that a human disease due to a hyperergic state to non-hemolytic streptococci may not be as specific as one due to direct infection with an immunologically specific bacterium.

[1] Derick, C. L., and Andrewes, C. H., PROC. SOC. EXP. BIOL. AND MED., 1925, xxii, 116.

[2] Andrewes, C. H., Derick, C. L., and Swift, H. F., *J. Exp. Med.*, 1926, xliv, 35.

[3] Derick, C. L., and Andrewes, C. H., *J. Exp. Med.*, 1926, xliv, 55.

3783

Immune Tissue Response to Non-hemolytic Streptococci.

HOMER F. SWIFT AND CLIFFORD L. DERICK.

From the Hospital of the Rockefeller Institute for Medical Research.

We[1] originally reported that if a rabbit had been recently inoculated by any route with streptococci of any type it would fail to show a secondary reaction following intracutaneous inoculation with suitable strains of streptococcus viridans. This might be due to one of two conditions: first a hyperergic, second an immune state. First, if due to a hyperergic state there might be a combination of accelerated hyperergic-secondary-reaction with the primary reaction; hence the initial response to introduction of the same sized inoculum would constantly increase up to a certain point. Second, if an effective immune state had been induced, the local protective mechanism against the inoculum might be so efficient that the sum of the primary and accelerated secondary reactions would be smaller than the initial response of a normal animal.

These two states were easily demonstrated in rabbits, according to the route used for inoculation. When repeated intracutaneous injections were made of 0.1, 0.01 and 0.0001 cc. at the time of each inoculation, the intensity of the local reaction at the site of each injection increased, up to a certain limit. Another group of rab-

bits inoculated intravenously showed very much smaller local reactions at the site of test intracutaneous inoculations. For example, at the site of a 0.0001 cc. inoculation there was only a slight erythema or a hard nodule 3 to 5 mm. in diameter, contrasted with a hard infiltrated papule 12 to 15 mm. in diameter, and 1 to 3 mm. in height in a hyperergic animal. In a highly immune rabbit the local reaction to 0.01 cc. was distinctly less than that of a hyperergic animal to 0.0001 cc. Immune animals have not developed interstitial keratitis following corneal inoculation, nor have they shown the lethal, tuberculin shock-like, reaction following intravenous injection.

The hyperergic state has been maintained or increased to a certain level by repeated intracutaneous inoculations or by the production of a subcutaneous lesion with an infected agar focus. When these hyperergic animals were inoculated intravenously with small doses of streptococci their condition was changed from a hyperergic to an immune state, while controls, not inoculated intravenously, remained hyperergic. In a few instances it was possible to convert an animal from the immune to the hyperergic state by discontinuing intravenous immunization and producing numerous local foci. Intravenous inoculation with strains of streptococci which were culturally and serum immunologically distinct from those used for the production of focal lesions resulted in the alteration from a hyperergic to an immune state in respect of the latter strains.

It appears, therefore, that both hyperergy and active immunity to non-hemolytic streptococci are not as specific as would be expected, *a priori,* from cultural and serum immunological differentiation. The induction of each state depends more upon the mode of inoculation of the animal than upon the specificity of the streptococci.

Our theory at present is as follows: Hyperergy to non-hemolytic streptococci is an early stage of resistance in which there is a maximal response of the tissues to a minimal stimulus; it is the result of the action of the antigen—the streptococci—in a limited area represented by the focus, where tissue destruction occurs. It may, indeed, depend upon substances arising in such a focus. Complete, or efficient immunity, on the other hand, is an optimal response of the tissues to a maximal—within certain limits—stimulus, and is the result of action of the antigen over a wide area without the induction of focal tissue destruction.

[1] Andrewes, C. H., Derick, C. L., and Swift, H. F., *J. Exp. Med.*, 1926, xliv, 35.

3784

Effect of Living Gall Bladder on Human Biliary Calculi.

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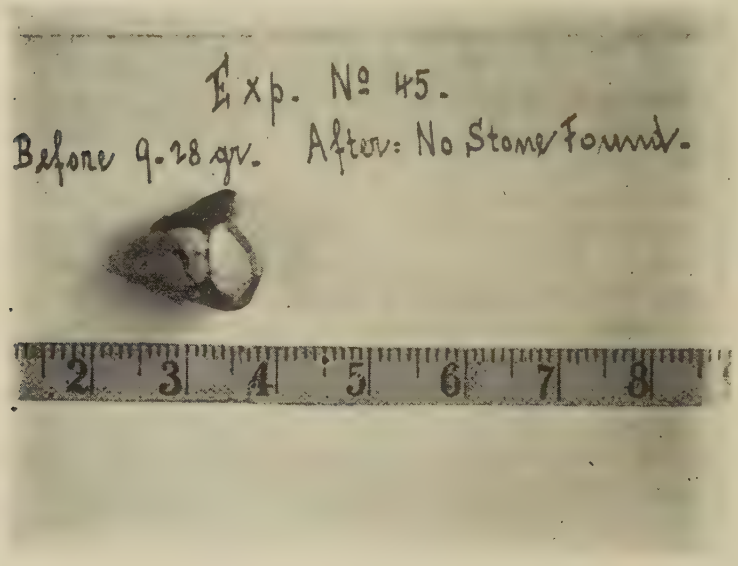
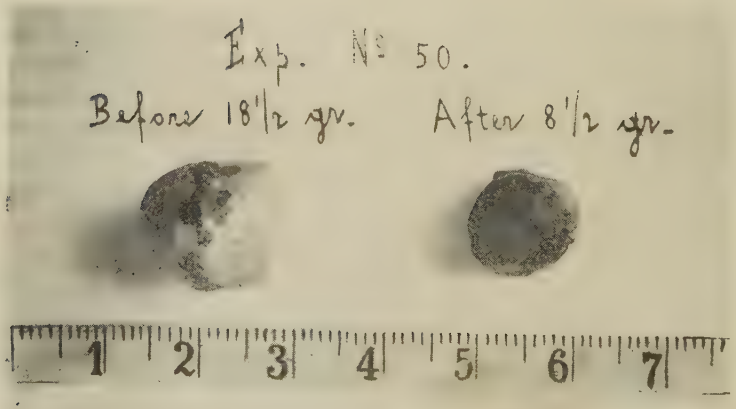
To determine the effect of the normal living gallbladder upon biliary calculi, the gallbladders of 8 normal dogs were opened under





surgical anesthesia and asepsis and stones from human bladders, too large to pass through the canine ducts, were inserted. The stones chosen were of the compact variety made up of bile pigments, calcium, iron, and, in some, cholesterol. The experimental animals were confined in laboratory cages and were given a regular mixed diet. After an average duration of 60 days in these 8 dogs, the stones had diminished in size in 3 and had disappeared in 5.

3785

Effect of Bacteriophage Upon the Agglutination of Hemolytic Streptococci.

GREGORY SHWARTZMAN.

From the Laboratories of the Mount Sinai Hospital.

There exists a definite effect of the bacteriophage upon the phenomenon of agglutination in the coli-typhoid-dysentery group, as is seen from papers by d'Herelle,[1] Bordet,[2] Gratia[3] and others. Since the studies on this problem were confined exclusively to the coli-typhoid-dysentery group, it was thought desirable to extend them to hemolytic streptococci in which the phenomenon of bacteriophage was shown to exist, Dutton,[4] Shwartzman.[5]

The effect of two lytic principles upon various strains of pathogenic streptococci which differed in their antigenic structure and their susceptibility to these principles, was investigated. The results may be grouped as follows:

1. The first group of changes of a whole culture was observed with a strain of scarlet fever streptococci grown in broth containing lytic principle. The changes consisted of complete inagglutinability by the normal culture serum; considerable loss of agglutinin absorbing power, and partial loss of agglutinogenic power. Two rabbits persistently immunized with this antigen responded with agglutinins of a low titer (1:400). The same method of immunization with the normal culture gave sera of titer 1:12,800.

2. When a strain highly susceptible to phage was made resistant to this principle it underwent changes which were still more marked than those described above. These changes manifested themselves in complete inagglutinability, complete loss of agglutinin absorbing and agglutinogenic properties. Five animals failed to respond to immunization with lysed cultures or with the resistant type of this

strain. Further attempts to obtain anti-sera should be made before complete loss of agglutinogenic properties can be safely accepted.

3. Examples of changes of a different character were afforded by representatives of scarlet fever streptococci and a strain of rabbit hemolytic streptococcus. These streptococci when treated by the phage became inagglutinable by homologous normal culture sera. However, the specific antigens were completely preserved, since the phage cultures were able to absorb agglutinins from normal culture sera, and incited the production of agglutinins for the normal cultures. Moreover additional components became prominent in the phage cultures. The phage cultures sera agglutinated the homologous as well as the normal cultures to a high titer. This was in contrast to the effect of the normal culture sera which were not able to agglutinate the phage cultures, or did it in a very low titer. The added components were related to the specific antigens of the normal cultures, from which they were derived. In one instance (rabbit streptococcus) the additional component was not related to the specific antigen of this strain.

The relation of the additional components to other strains was studied by cross absorptions and cross agglutinations with various strains on hand. It was found that these components were of the "group" variety and thus considerable cross agglutinations occurred with heterologous strains.

4. Two strains of streptococci (one green producing strain and one pyogenes hemolytic streptococcus) underwent a complete modification. The changes consisted of complete transformation of the normal cultures into antigens of entirely new specificity.

It appears that the bacteriophage phenomenon may play an intricate rôle in the serological grouping of various strains of pathogenic streptococci.

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[1] d'Herelle, The Bacteriophage, English edition, 1926.

[2] Bordet-Cinca, *Compt. rend. Soc. Biol.*, 1921, lxxxiv, 280.

[3] Gratia, A., *J. Exp. Med.*, 1922, xxxv, 287.

[4] Dutton, L. O., *J. Inf. Dis.*, 1926, xxxix, 48.

[5] Shwartzman, Gregory, *J. Exp. Med.*, 1927, xlvi, 917.

3786

The Effect of Iodine on Creatinuria in Hyperthyroidism.

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The urine of normal human adults taking a diet free from meat, fish, peas and beans, contains no creatine. The only known exception to this is in women during the menstrual period when small amounts of creatine may appear in the urine. A very high creatine-free protein diet may produce creatinuria in women but has not been obtained in men. Creatinuria has been observed in starvation, wasting diseases generally, during carbohydrate deprivation, diabetes mellitus, Graves' disease, fevers and the muscular atrophies and dystrophies.[1] Studies made on 21 cases of Graves' disease with or without a nodular thyroid gland confirm in general previous observations regarding the appearance of creatine in urine of individuals with hyperthyroidism.

In observing the effect of iodine on creatinuria in hyperthyroidism the cases have been studied in a specially organized metabolism ward under rigid metabolic conditions. Each patient was given a creatine-free diet with a caloric value equivalent to 100% above the actually determined basal requirement, with a protein intake of 1½ gm. per kilo and the carbohydrate and fat distributed according to the likes and dislikes of the individual. Usually, however, the carbohydrates were taken more freely than fats. Such a diet brings the patient into nitrogen equilibrium within a few days. After three days to a week Lugols solution, 1 to 3 cc., is given, in most instances as part of the program in preparation for operation.

Out of 16 cases of true Graves' disease all except one have shown a daily excretion of creatine varying from 200 to 1000 mg. On the exhibition of iodine in the form of Lugous there is a striking diminution of creatine excretion to less than 100 mg. This is usually accompanied by an improvement in symptoms and a fall in the basal metabolism rate. In one case there was no improvement in symptoms or fall in basal but a remarkable drop in creatine excretion occurred. Of the 5 cases with nodular thyroid glands, the so-called toxic adenomas, relatively small amounts of creatine in the urine were observed although the basal metabolism was high in some of the patients. Iodine in the cases of toxic adenoma studied seems to have only a slight influence on the general condition and almost no

effect on the creatinuria. While the observations are by no means extensive, the creatinuria occurring in fever (pneumonia, sepsis) and experimental hyperthyroidism are uninfluenced by iodine. The result so far as it concerns experimental hyperthyroidism might be anticipated, since the symptoms, pulse rate, and basal metabolism produced by intravenous injections of thyroxine in rabbits are not modified by the administration of iodine by mouth.[2]

The significance of the observations reported in many respects is not clear. The fact that iodine diminishes the excretion of creatine in Graves' disease and has no effect on experimentally produced hyperthyroidism lends further support to the idea that the effect of iodine is due to a congestion within the thyroid gland mechanically interfering with discharge of thyroxine into the general circulation.

[1] Hunter, Andrew, *Physiol. Rev.*, 1922, ii, 586.

[2] Sturgis, C. C., Zubiran, Salvador, Wells, Guy W., and Badger, Theodore, *J. Clin. Invest.*, 1926, ii, 289.

3787

Allergic Reactions in Rabbits to Bacterial Antigens.

FRANKLIN M. HANGER, JR. (Introduced by W. W. Palmer.)

From the Department of Medicine, Presbyterian Hospital, Columbia University.

In January, 1927, Mackenzie and Hanger[1] described reactions in the human, following intradermal injections of antigens prepared from streptococci obtained from throats of rheumatic patients and normal individuals. These antigens were thermostable and non-neutralizable by immune serum. Like tuberculin, they gave a negative reaction during most acute infections, and in very young children. It was assumed by us that the chronic lesions, produced in the nasopharynx and tonsils of even normal individuals by the streptococcus, render such persons allergic to the bacterial protein as is manifested by these skin reactions. When these same streptococcus antigens, strongly active for the human, are injected into the skin of normal rabbits, the animals seldom show a local response. Cultures were therefore made of the naso-pharynx of rabbits to determine the presence or absence of the streptococcus, and also to establish the general character of the bacterial flora in these animals.

Our results agree essentially with those of Webster[2] and of Bull,[3] who note the overwhelming predominance of gram negative organ-

isms, among which, members of the *B. lepisepticum* group are almost a constant finding. Webster has shown[4] that this group is responsible for snuffles and most of the other chronic respiratory infections in the rabbit. It seems reasonable to assume that in this animal the rôle played by *B. lepisepticum* is quite analogous to that of the streptococcus in man, and that the rabbit should show allergic reactions to antigens prepared from these organisms. Antigens were prepared from a number of strains of *B. lepisepticum* and *B. bronchisepticus*, isolated from the naso-pharynx of rabbits in our laboratory, among which sporadic cases of snuffles had appeared. Also from the virulent Rockefeller strain (R.D.) kindly furnished us by Dr. Webster. The method consisted of preparing Berkefeld filtrates of 72 hour plain broth cultures and preserving in sterile stoppered bottles in the ice box. Adult rabbits were shaved over the flanks and abdomen, and 0.2 mls. of the undiluted filtrate injected into the skin. Within 5 hours a slightly raised area of erythema appeared, which reached greatest intensity in about 24 hours, and usually faded within 60 hours, leaving a scaling, slightly pigmented spot. The animals varied much in the intensity of the reaction. In the strong reactors, the area was 5 to 7 cm. in diameter with considerable swelling and even ecchymosis. Most of the animals showed an erythema 3 to 4 cm. in diameter, while a small proportion showed no reaction, although they were found to harbor *B. lepisepticum* in the naso-pharynx. Striking immunological differences were noted between the "Strong" and "Weak Reactors," which will be recorded elsewhere.

Similar tests were carried out in guinea pigs, and the skin reactions were irregular or negative. These animals are quite free of chronic upper respiratory lesions and show a most inconstant bacterial flora.

Many factors influenced the intensity of the reaction though it remained strikingly constant for the same rabbit. In the young under 3 weeks of age, it was absent, but usually appeared by the 6th week. During pregnancy and lactation, wasting disease and severe acute infections, it was markedly diminished. Intravenous injections of the filtrate or of bacterial suspensions or even of India ink caused it to disappear for several days, or permanently, if the animal became ill from the injection. There was a diminishing effect of successive intravenous injections on the intensity of the skin reactions.

Repeated injections of the filtrate had but little effect. Animals which received 20 or more mills during the course of an experiment showed no change in their reactivity. It was also impossible

permanently to alter the reactivity of a section of skin. When repeated injections were made into an area already inflamed, there was less effect than usual, apparently because the endothelium was incapable of taking up more of the antigen. After healing, however, the reactions resumed their former intensity. The potency of the filtrates was somewhat diminished by boiling for one hour. We were unable to neutralize it with immune serum of an agglutinin titer 1:40. No deterioration has been noted in filtrates kept at ice box temperatures for 6 months.

The skin test is not limited to any strain of organism. Filtrates of most of the gram negatives from the pharynx of the rabbit will produce it, though certain of the avirulent ones lost this capacity after cultivation for several months on artificial media. Antigens from the virulent Rockefeller strain (R.D.) were active for all groups tested; however, the intensity of skin reaction is not a criterion of virulence, since strains of relatively harmless *B. lepisepticum* isolated from a certain group of rabbits, produced filtrates even more potent for those particular animals than those obtained from the more invasive organism. Broth controls were uniformly negative.

There is apparently considerable antigenic relationship between many gram negatives of different biological groups, inasmuch as rabbits react to filtrates of *B. influenzae, B. coli* and meningococcus organisms, to which they could not have been directly sensitized. Likewise, humans who react to filtrates of these organisms also react to those of *B. lepisepticum.*

[1] Mackenzie, G. M., and Hanger, F. M., *J. Immunol.*, 1927, xiii, 41.
[2] Webster, L. T., *J. Exp. Med.*, 1924, xxxix, 843.
[3] Bull, C. G., and McKee, C. M., *Am. J. of Hyg.*, 1927, vii, 110.
[4] Webster, L. T., *J. Exp. Med.*, 1926, xlviii, 573.

3788

Phagocytic Activity of Capillary Endothelium of Skin and Probable Relation to Focal Immunity.

FRANKLIN M. HANGER, JR. (Introduced by W. W. Palmer.)

From the Department of Medicine, Presbyterian Hospital, Columbia University.

Bacteria and many colloidal substances injected intravenously rapidly disappear from the blood stream. This is due chiefly to the phagocytic activity of the reticulo-endothelial system of the liver,

spleen, lungs and bone marrow, where the foreign particles may be demonstrated within the cells. The endothelium of other organs such as the kidney, muscle and skin, normally lack this function. Experiments are here reported which demonstrate a temporary phagocytic acitivity of the capillary endothelium of the skin, following injury of various sorts. This activity appears almost immediately after the trauma and disappears within an hour, and constitutes, perhaps, the initial stage of inflammation.

A rabbit that had previously been found allergic to certain bacterial filtrates was selected. Sterile filtrate of *B. lepisepticum* (0.2 mil.) was injected intradermally at hourly intervals along the previously shaved side of the animal. After 5 or more hours, erythema appeared at the sites first injected, while the more recently injected ones showed nothing after the absorption of the filtrate. Diluted India ink (2.5 mil. of Higgins waterproof and 2.5 mil. of normal saline) was then injected intravenously. Almost at once, the site of the most recent intradermal test became dark in color, while the normal skin and the older injection sites remained unchanged. Twenty-four hours later, no erythema was present in the injected sites where ink had been deposited, but wherever no deposition had taken place erythema had developed. Microscopic examination of the skin shows ink particles filling the endothelial lining of the capillaries and the reticular cells, but none in the tissue spaces or wandering cells. This ink deposit persists during the animal's lifetime. The reaction is not specific. Plain broth, washed bacterial suspensions and simple trauma, such as scarification, will often produce it, but rarely as intensely as an active filtrate. Repeated trials show that the most active phagocytosis occurs in areas stimulated about 15-30 minutes before the ink injection. The reaction rarely occurs after 1 hour.

A few experiments have been done where the India ink was diluted with distilled water instead of normal saline. In these animals there was apparently considerable laking of cells, for the sites became bright red and there was no evidence of ink phagocytosis. Attempts have been made to demonstrate similar phagocytosis for bacteria by using tubercle bacillus and staphylococcus instead of ink, but apparently the reaction is confined to foreign bodies in solution, or in a very finely divided state.

Up to the present time, we have failed to elicit the phenomenon in the capillaries of muscle or kidneys. This requires further study for the experiments were done under ether anesthesia, which usually inhibits the reaction.

The reaction can only be elicited in rabbits capable of giving positive skin tests to bacterial protein. We have shown in another series of experiments that it is these strong reactors who resist infection when virulent *B. lepisepticum* organisms are injected intradermally, while the negative reactors usually succumb to a rapidly spreading infection. It appears that this prompt activity of the endothelial cells is related to the focal immunity of the animal.

3789

Some Results of the Application of High Pressures to the Heart.

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Localized pressure applied to muscle or nerve is commonly employed to depress, or abolish function in these tissues. In the present experiments our purpose is to observe the effects of high pressure upon the heart when applied in a manner so as to give equal compression throughout the organ.

A special compression chamber has been constructed which is capable of withstanding pressures of 80 atmospheres, and is large enough to admit a small optically recording membrane manometer with a frog, or turtle heart attached. The heart and optical manometer are completely filled with Ringer's solution and mounted in the compression chamber which is also completely filled with the solution. In this manner the heart is surrounded inside and out with the Ringer's solution, and the pressure when applied to the heart is through this liquid medium.

Since the source of pressure in our experiments is nitrogen gas, provision is made also, for the liquid system of the compression chamber to be continuous through a small bore tube to a reservoir of about 100 cc. capacity. The reservoir is partly filled with Ringer's solution, and from the top of it a small tube leads to a high pressure nitrogen tank. In one end of the compression chamber a small window, backed with heavy plate glass, provides a way for recording the movements of the manometer system produced by the contractions of the heart.

When pressure of about 60 atmospheres is applied to a normal beating heart, an increase of nearly twofold is produced immediately

in the amplitude of its contractions. The maximum augmenting action of compression is not shown in the first cycle after the onset of pressure, but usually it requires from 3 to 5 cycles for the full effect to take place. This delay does not appear to be related to the manner of introducing the pressure, since it occurs similarly in instances where the full 60 atmospheres is turned on almost instantaneously. If the pressure is maintained on the heart the records show a gradual decline in the height of the contractions. With release of the pressure the effect on contraction is equally striking but opposite in character—the contractions show a sudden falling off to an amplitude considerably below the pre-pressure level. There follows a period of recovery which varies in degree in different experiments, but commonly the records show a complete return to the pre-pressure amplitude. The changes in contractility accompanying the application and removal of pressure suggest that the nature of the effect of compression is a physiological stimulation, and not due to purely mechanical factors.

Hearts beating normally by their own automaticity show a definite increase in rhythm immediately following compression. The increase in rate is most apparent in the series of cycles immediately after the onset of pressure, but following this a gradual slowing of the rate is shown while the compression is maintained. When the pressure is released the rhythm shows a sudden marked slowing, but usually returns again approximately to its original rate within a period of approximately five minutes. In some experiments preparations were used which exhibited partial heart block. Under compression the condition of block is relieved and a normal rhythm established, indicating that pressure has a beneficial effect on conductivity.

3790

Studies on Crown Gall Transplants.

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That great difficulty is experienced in transplanting spontaneous animal tumors to other animals of the same species is well known. Yet, transplanted animal tumors once established may be transferred from animal to animal of the same species for many years with con-

siderable ease. The transplants take approximately in all cases.

Jensen claims[1] that successful transplantations of spontaneous and artificially produced crown galls of the sugar beet may be made on the sugar beet without stimulating the host tissue itself to crown gall formation.

The present report deals with the inoculation of young parts of tobacco, tomato, geranium, sugar beet, yellow and red mangels, rubber and castor bean plants with pieces of crown gall tissue arising on similar plants from inoculations, with virulent strains of *Bacterium tumefaciens.* Pieces of crown gall tissue of *Ricinus communis,* when transplanted into the same and other *Ricinus* plants, produce small crown galls in all cases. Transplants of the growths of the second generation to other plants were also found to be effective in producing crown gall tissue. These new growths, however, were generally much smaller than those produced by the primary inoculation with the bacterium or with the crown gall inoculum.

Ricinus crown gall tissues transplanted to growing portions of the tobacco plant were also found to produce galls. Similar results were obtained with crown galls of tomato, geranium and the beet. These neoplasms, however, are not conclusively of the inoculum type, but are generally of the host type, for, while it is impossible to distinguish *Ricinus* crown gall on tobacco from tobacco crown gall, it is clear that these crown galls are strictly of tobacco type and origin. The crown gall tissue on the tobacco became differentiated and formed leafy shoots with characteristic tobacco leaves.

In the majority of cases, observed in these studies, the crown gall inoculum invariably dried up and died 3 to 4 days after the transplant was made, yet in actively growing plants, which received the inoculation, swellings were observed from 7 to 21 days later, and well established crown galls, although small, were to be seen about a month after the transplant was made. This occurred whether homeo, auto, or heterotransplantation was made.

The transplantation of small maroon colored pieces of sugar beet crown gall induced by *B. tumefaciens,* to the yellow colored mangel ("yellow tankard"), produced a relatively large number of yellow pigmented crown galls, and in a small number of cases only, there was indisputable evidence of the growth of the inoculum as shown by the presence of maroon colored crown gall on the yellow root. However, in all cases, there were also distinct proliferations of the host, as shown by gross sections of the tumor. Microscopical examination of the tissue after the usual fixation methods, fail to

show any difference between them. There is, however, a distinct proliferation of the host tissue.

These studies show that the crown galls are not generally formed as the result of the growth of the transplanted tumor tissue itself, but are produced as the result of the changes which succeed the introduction of *B. tumefaciens* with the inoculum. Plate cultures of parts of the crown gall tissue from which inocula were taken, always yielded an abundance of *B. tumefaciens* as determined by cultural studies, smears, and subsequent inoculations.

[1] Jensen, C. O., *Meddelelser fra den Kgl. Veterinaer—og Landbohjskoles Serumlab.*, 1918, liv, 91-143.

3791

Demonstration of a Toxin in Cases of Pemphigus.

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Pemphigus is a skin disease of a very grave nature, often leading to a fatal outcome. There is practically nothing known concerning its etiology. In connection with a routine examination of various pharmacological properties of blood samples obtained from a variety of skin diseases, the authors have discovered certain properties characteristic of the blood of pemphigus patients, which point to the presence of a toxin in that disease and which throw light on the etiology. One of the authors has already pointed out that by means of phytopharmacological methods it can be demonstrated that the blood serum from patients suffering from pernicious anemia is very toxic for plant protoplasm.[1] This reaction of pernicious anemia blood is very characteristic and is not given by the blood from other cases of anemia, leukemia, etc., so that a differential diagnosis can be made by using the phytopharmacological test. An examination of blood serum from 8 cases of pemphigus has revealed that such serum is also definitely toxic for plant protoplasm. The toxicity, however, is of a different nature from that shown by pernicious anemia blood. The experiments were made by studying growth of seedlings of *Lupinus albus* suspended in a nutrient plant physiological solution, and in the same solution plus 1% of pemphigus serum. The phytotoxic index of 8 cases gave an average of 56%. This figure is a

little higher than the average figure obtained for pernicious anemia serum in which the average was found to be well below 50%. The serum from pemphigus patients was different in its toxicity from that of pernicious anemia in certain other respects, also, which will be described in a fuller paper. An examination of the sterile fluid obtained from the bullae showed that it was also toxic for plant protoplasm. A specimen of blood obtained from a pemphigus patient shortly before death revealed an increased toxicity. Subjoined table gives the separate data obtained in individual cases.

TABLE—*Phytoxic Index.*

Case Number	Normal Serum	Pemphigus Serum	
1	70	55	
2	72	53	
3	70	62	Convalescing case
4	75	58	
5	70	48	Moribund case
6	74	52	
7	72	57	
8	75	60	
	Average 72	Average 56	

[1] Macht, D. I., *J. Am. Med. Assn.*, 1927, lxxxix, 753.